

Another sort of braindrain

The steady drift of young scientists away from many rural and even urban areas in the UK must be stopped.

One of the easier things for the delegates to UNCSTD, the United Nations Conference on Science and Technology, to decide upon in Vienna recently was that the flow of qualified personnel away from developing countries is one of the most serious obstacles to development. It was equally easy to enjoin developing countries to create the appropriate work environment, and the developed countries to go steady on their recruiting from the Third World. Whether these fine words make an iota of difference to the brain drain is, of course, another matter. And, apparently for political reasons, one type of brain drain — from one developing country to another — was discreetly not mentioned in the Vienna documents, although it is growing in size and could potentially do considerable damage by the syphoning off from one country to another of people with skills of particular relevance to development. Not all the brain drain problems exist in the Third World, however. First World countries can suffer from less publicised but equally disturbing movements of qualified people, and inadequate attention is paid to this problem.

In Britain, as in many other countries, every young person with at least a passing interest in science will certainly be able to study the rudiments of physics, chemistry and biology. In every school with any pretensions to academic quality at all, those who wish to pursue this interest in more detail will be able to concentrate on the sciences, which may additionally include subjects such as geology and psychology, up to the level of university or polytechnic entrance. At this stage, however, for reasons of convenience, the process of concentration starts, and the next three years have to be spent in one of the hundred or so cities in which facilities for higher education exist.

What happens upon graduation? Most but not all of those with scientific qualifications will gravitate towards the academic and teaching world, towards the civil service or towards science-based industry. As far as school-teachers are concerned, opportunities exist, of course, anywhere within the UK. But this is far from true for most other forms of employment. The curious reader might take a list of scientific jobs available outside school-teaching and plot their location on a map. The chances are quite

strong that a circle of radius 100 km centred on London will encompass at least 60 per cent of all the vacancies. The chances are equally strong that the number of openings north of the Trent will be less than 20 per cent of the total available.

Scientific jobs in Wales, Scotland and Northern Ireland are particularly rare, as are those in relatively rural parts of the country. But a matter of considerable interest is that jobs in many large cities which achieved their prosperity through industry in the nineteenth century are also relatively uncommon. South-East England is by far the most fertile field for scientific employment in the UK, as it is for many other types of white-collar employment.

Does this matter? In some ways it is a good thing if it ensures a critical mass of scientific expertise capable of sustaining the UK's position in the face of international competition. But whilst there are benefits to be derived from concentration, there are also serious disadvantages. If bright young scientists from large areas of the UK cannot find satisfactory employment in their home area but have to migrate to better-endowed areas, there comes a time when the school-teachers who have been diligently educating these students only to see them all disappear begin to lose heart, and science is listlessly or even badly taught. There comes a time when the scientific awareness of whole regions of the country declines because there are inadequate numbers of scientists around to sustain it. There eventually comes a time when good potential scientists are lost in large numbers.

Centres of excellence are undoubtedly desirable in some academic disciplines, and it would be folly to disperse, say, particle physicists to all corners of the country. But much science-based industry, and many civil-service establishments do not really need to be within close reach of London to be effective; their presence in regions at present less highly populated with scientists might even be of more value than being within an hour or two of the capital city.

There is no obvious time to grasp this problem; the brain drain is slow and makes few headlines. But unless there is careful study of the problem soon, followed by corrective action, the pace of the migration will continue to grow to the detriment of large areas of the country. □



US expected to exempt most recombinant DNA experiments from federal regulation

Eighty-five per cent of US recombinant DNA experiments will be exempted from controls, if the recommendations of a national committee are accepted. **David Dickson** reports

A large proportion of current experiments using recombinant DNA techniques is expected to become exempt from federal regulations covering such research, following a recommendation which a national advisory committee agreed last week to make to the director of the National Institutes of Health.

In a split decision, the NIH's recombinant DNA Advisory Committee agreed that all experiments using the 'disabled' K12 strain of the bacterium *Escherichia coli* be exempted from the guidelines, except for those which are known to present a particular type of hazard.

The decision has been taken after pressure from scientists, many of whom have argued that the results of recent research fail to support earlier speculations over the hazards of recombinant DNA research. Critics, however, still argue that such a decision is premature, and that there is inadequate knowledge of the risks involved to justify taking such a significant step at this stage.

In its recommendation, the RAC suggests that the experiments exempted from the guidelines should still be carried out under P1 containment conditions, the lowest of four levels of physical containment established in the guidelines which were first published by the NIH in 1976. It also recommends that the experiments should be notified to local institutional biohazard committees, which all institutions carrying out recombinant DNA research on federal funds are required to set up.

However under the committee's recommendations, which are expected to be accepted with little alteration by Dr Donald Fredrickson, director of NIH, there will be no need to obtain prior approval for the experiments from the local committee. Nor will it be necessary to inform the NIH's own Office of Recombinant DNA Activities (ORDA), as all research workers are required to do at present.

Members of the advisory committee voted by ten votes to four, with one abstention, in favour of the exemptions. In general, the scientific members of the committee, many of whom are actively involved in recombinant DNA research or related fields, voted in favour of the exemptions — while those appointed as 'public interest' representatives on the committee voted against them.

During the debate on the proposal, it was said that about 85% of current recombinant DNA experiments were conducted using the *E. coli* K12 strain. The

proposed exemptions would not apply to experiments currently prohibited under the guidelines, such as those involving the transfer of genetic material from known toxins, or to experiments involving more than 10 litres of culture — for which special permission is required.

Some members of the committee argued that no decision should taken before the committee had had time to examine the implications of the risk assessment scheme which has been developed by Dr Sidney Brenner of the Medical Research Council laboratory in Cambridge, England. This approach is now being used as a basis for decision-making by Britain's Genetic Manipulation Advisory Group. However other committee members argued that Dr Brenner's concerns were adequately covered by the NIH guidelines. And it was also decided that it was not necessary to wait until Dr Brenner could appear personally before the committee before recommending exemption for the *E. coli* K12 experiments.

During the committee's two-day meeting in Bethesda, approval was given to a set of procedures which have been drawn up by the director of NIH under which private companies can voluntarily register their experiments with ORDA.

Dr Fredrickson told the committee that he was confident that commercially sensitive information could be adequately protected, a factor that had previously

made companies reluctant to divulge details of their proposed experiments. He also said that before leaving the Department of Health, Education and Welfare, ex-secretary Mr Joseph Califano had indicated that he was opposed to new legislation covering the private sector.

In a subsequent closed session, the committee gave its approval to large-scale experiments involving more than ten litres of culture submitted by the pharmaceutical company Eli Lilly and the west coast research company Genentech. The two companies are currently collaborating on efforts to produce sufficient quantities of human insulin to begin clinical trials.

The committee also agreed on a set of procedures, based on proposals of a small working group, on how it should conduct its business. Against the advice of the NIH's legal counsel, Mr Richard Riseberg, the committee decided that members having a 'personal stake' in the outcome of a particular line of research should not necessarily be excluded from discussions related to that research.

Mr Riseberg informed the committee that it might be contravening federal law if, as an advisory committee, members took part in such discussions. Some committee members, however, pointed to the difficulty of determining what 'personal stake' meant in the context of scientific research — and the committee decided to strike out the provision. □

US to harmonise remote sensing

The United States has agreed to initiate efforts to 'harmonise and rationalise' international activities in the field of remote sensing — the mapping of natural resources from satellites. Such efforts should, according to the US, have two main goals: ensuring the compatibility between the remote sensing systems now being developed by different industrialised countries, and stimulating the full use of remote sensing data by the developing countries.

The decision to take this initiative was announced during the first week of the United Nations Conference on Science and Technology for Development held in Vienna last month, by Father Theodore Hesburgh, head of the US delegation to UNCSTD. Addressing the plenary session of the conference, Father Hesburgh said that the US proposed bringing together the operators of remote sensing satellites, as well as the users, to develop an international system.

"We believe that satellites should be

operated so that all can have easy access to the data and so that information can be collected without unnecessary duplication and for maximum mutual benefit", Father Hesburgh said.

"The objective is to ensure developing countries improve their access to information for the use and management of forests, rangelands, water supplies, soil preservation, and the identification of new mineral and water resources."

The Administration has been under pressure from Congress for some time to decide what steps to take following the experience gained with the series of Landsat experimental satellites. Landsat A was launched in 1972, and operated successfully for five years, and its successor Landsat B was launched in 1976. The last of four satellites, Landsat D, is due to be launched in 1981.

Despite the exceptionally high quality of the Landsat images, analysis of them had had mixed success. In some areas, such as the survey of water or mineral resources,

the satellites have produced a large yield of high quality information. In others, initial goals have proved to be over-optimistic. In particular, it has proved far more difficult than predicted to use satellites for assessing agricultural yields — for example because of problems in distinguishing between different types of crops — and more research is now being done in this area.

Enough information has been gained so far, however, to convince the US that remote sensing can provide useful information to developing countries in fields from water resource engineering (such as building dams or irrigation systems) to mineral prospecting. The US Agency for International Development (AID) has already carried out research projects and training in more than 30 countries, demonstrating how Landsat data can be used by local planners and policy-makers.

Speaking in Vienna last week after Father Hesburgh's address, Mr Tom Pickering, director of the State Department's Bureau of Oceans and the Environment, said that in announcing its new initiative, the US had two main goals, both of which were intended to help

developing countries make better use of remote sensing data.

The first goal is to bring together the various countries now planning their own satellite systems to ensure the maximum possible complementarity and compatibility. The aim, for example, would be to ensure not only the minimum of overlap and duplication, but also that wherever possible a developing country receiving station could use the same equipment for receiving data from different satellites.

The second goal is to bring together, possibly on a regional basis, those who intended to use satellite data to obtain information about their own resources, and how these might best be managed. "Our hope is that through discussion with users we can define together the sort of data that can be most effective in answering their needs, and in discovering about their own territories the type of things that they would like to discover," Mr Pickering said.

The Administration's decision was welcomed by Senator Adlai Stevenson Jr, chairman of the Senate's Science and Space Subcommittee, who has long been a critic of the current Administration's lack of plans for an operational Earth-monitoring

satellite system, advocating this as one of the most important areas in which US technology can help developing countries.

Describing the wide potential applications of remote sensing, Mr Stevenson said that the US "was concerned that these technologies will follow many national courses and that, if some cooperative steps are not taken soon, it may be too late to at least develop complementary technologies. By complementary we mean not only the physical systems, but also in their operation, so that satellites can be used in a way that avoid redundancies and maximises knowledge of the resources of the world."

Backing up the proposal made in Father Hesburgh's speech, AID mounted an exhibition in Vienna demonstrating the type of data that can be derived from Landsat and other satellites, and the way in which it might be used. The exhibition was to have been entitled "remote sensing: an appropriate technology for economic development" — until advocates in AID of a less capital-intensive approach to Third World needs objected, and the word "appropriate" was dropped from the title.

David Dickson

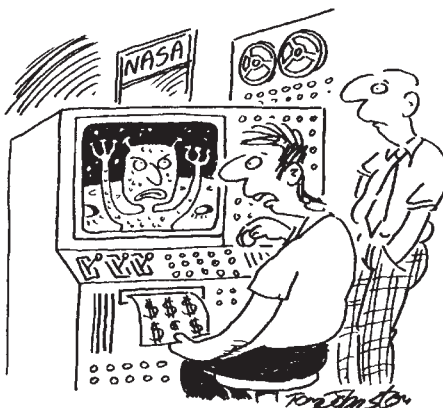
Interference with data from Titan

Vital data on the surface temperature of Saturn's giant moon Titan may have been lost as the result of radio interference during the transmission of the data from the Pioneer 11 spacecraft to the National Aeronautics and Space Administration's Ames Research Center in California last week. In place NASA has a series of dollar signs, printed by the computer receiving the data as an indication that it was unintelligible.

At first it was thought that all the relevant data, which came from readings made by an infrared radiometer aboard the spacecraft and would provide important clues about the planet's atmosphere, had been lost. However subsequent investigation showed that the data had been received — but in a highly garbled form. NASA scientists are now trying to unscramble what they can; they are confident that some of the information can be retrieved, but remain uncertain about its quality.

"The data was ratty, make no mistake about that. But it's there, and it may all be there, all 20 minutes of it, ground up in some scrambled data that was fouled in the communication link between Madrid (one of the three receiving stations for Pioneer transmissions) and California," mission chief scientist Dr John Wolfe said last Thursday.

Late last week, the precise cause of the radio interference was still uncertain. Initially it was stated that the interference had been caused by a Soviet satellite in Earth orbit, Cosmos 1124, which NASA



"It's the Titans! They say if we want to take pictures we can pay for them!"

officials had forgotten to ask Soviet scientists to switch off during the period in which the Pioneer data was being transmitted.

On Wednesday, the space agency issued a statement identifying the satellite as the cause of the problem. The statement continued: "NASA officials indicate that they have no doubt that the Soviets would have avoided the conflict on Monday, if they had been asked. However the impact of the potential interference was not recognised in time to make an additional request to the Soviets."

By the next day, however, NASA had changed its mind about the cause of the interference. Rather than the Soviet satellite, they now blamed the combined effect of a solar storm and low quality land transmissions between Spain and California — an explanation which had been put forward at the very beginning, but rejected when the Cosmos satellite's involvement had become known. □

Bulgaria plans to save energy

During the next Five Year Plan (1981-1985), all Bulgarian "energy consuming enterprises" will become subscribers to *Promishlena Energetika* (Industrial Energy), an organisation established this summer and described by Energy Minister Nikola Todoriev as "a scientific production combine". The main purpose of *Promishlena Energetika* is the saving of all forms of energy across the economy; its activities range from the reconstruction and modernisation of existing installations to an extensive research and development programme.

What precise form this research would take, the Minister did not specify. He was addressing a symposium on "Fuel and Energy saving and new energy resources" held in conjunction with the annual Plovdiv trade fair. Scientific seminars at the Fair were introduced some ten years ago, and, according to Penko Penkov, Chairman of the Bulgarian Chamber of Commerce, are the response of the organisers to the information explosion.

"Rapid technical progress makes us organise scientific and technical symposia", said Penkov, "in order to give experts from many countries a chance to keep up with the latest developments". This year, there were no less than twelve day or half-day seminars on subjects ranging from ergonomics, geodesy, and the stimulation of scientific creativity to textile and leather technology and the bacteriology of yoghurt. The three day energy symposium, however, with its massive foreign participation and the full

panoply of simultaneous translation, undoubtedly dominated the scientific events.

Foreign participation, indeed, was the major feature of the symposium. Apart from the Minister's speech, there were four Bulgarian contributions (on geothermal and solar power, hydrogen fuel and secondary power sources). Otherwise, with the exception of one Polish contribution, all papers were contributed by Western experts, ranging from nuclear to hydraulic power sources, and from general policy to specific devices.

This willingness to borrow Western achievements is nothing new to Bulgaria. One exhibit at Plovdiv — that of the *Progress* organisation, established last year to help bridge the gap from research and development to large scale implementation — carried as its motto a quotation from Mstyslav Keldysh, late President of the Soviet Academy of Sciences, that it is not the nation which

makes the first breakthrough who enjoys the benefits of a discovery but the one which implements it properly.

In 1976, said the Minister, when Bulgarian energy policy was revamped with the establishment of the National Energy Complex, Bulgaria had reached the position of one of the most developed nations in the world "in the energy sector at least" having increased specific consumption per head from 9% below world average in 1953 to twice the world average.

The new policy took a three-fold approach, stressing increased exploitation of local resources (mainly lignite), an expansion of nuclear generating capacity (which will reach 35% of the total generating capacity by 1990, with subsequent introduction of nuclear district heating for Greater Sofia) and a major programme of energy-saving.

In spite of massive efforts at economy by industry and domestic consumers alike,

these "passive measures" are not considered to be sufficient. Modernisation of existing plants, training of personnel to get the best use from their apparatus, and even the introduction of automated control systems for energy-intensive processes will all peak by the mid 1980s — leaving further gaps to be filled. These, stressed the Minister, can only be satisfied by new technologies.

Some of these will, of course, be of Bulgarian origin. Todoriev mentioned a new Bulgarian scheme, already in operation at the Maritsa-Istok-3 power station, which allows lignite to be burned without predrying — giving an overall fuel saving of some 5%. Others, including solar energy, the use of the geothermal waters of the Rhodope mountains, and the pressing problem of secondary energy resources, may well, if one judges from the Plovdiv seminar, rely considerably — at least in the early stages, — on foreign know-how.

Vera Rich

Pugwash calls for new efforts to control chemical weapons

The Pugwash Workshop on Chemical Warfare has joined impatient voices calling for progress in negotiations on a chemical weapons (CW) disarmament treaty. At a meeting in Stockholm recently, the Workshop urged that the current situation in the negotiations be re-appraised for the Biological Weapons Convention Review Conference, scheduled for 1980. The Review Conference is charged with assessing progress in CW disarmament as well. Most members of the Pugwash group also advocated the reopening of multilateral negotiations on a CW treaty, for there is a major weakness in the Biological Weapons Convention is that it allows research into new biological agents for 'defence'.

Since 1976, the US and the USSR have been having secret talks about chemical weapons disarmament — a subject earlier discussed at the multilateral Geneva Disarmament Conference. But the superpowers' bilateral talks have made no progress. They have instead taken the wind out of the Geneva negotiations, and many of the delegates to the Disarmament Conference have expressed themselves impatient with the stagnation. For example, the leader of the Swedish delegation, Mrs Inga Thorsson, tried recently to move things along when she suggested that the conference should begin its own CW negotiations in parallel with the superpowers. The Pugwash group urged the reopening of multilateral negotiations irrespective of the state of the bilateral talks.

A CW capability consists of different elements: laboratories for research and development, facilities where the weapons are made, and facilities where they are stockpiled. Any treaty aiming at CW

disarmament must deal with all these different elements. The Workshop considered them in turn, asking for each whether it would be better to destroy it or convert it to civilian use, and how such measures could be verified.

Pugwash, a private affiliation of scientists interested in disarmament questions, is not an official government body, but it provides a useful arena for its members from east and west to test ideas and atmospheres. The temperature of the official negotiations can be said to be above freezing simply because the Pugwash group is still functioning. At this meeting, the 30 participants produced nothing dramatic; but some of them (who preferred not to be named) were prepared to point to parts of its report with cautious optimism.

It was, for example, said to be a hopeful sign that the report contains a list — agreed by both sides — of six infrastructural recommendations for the verification of the destruction of declared stockpiles. Perhaps the most interesting of these points are that whatever international body is established to verify the destruction, it should have an independent technical capability, and that continuous presence of monitoring personnel at the destruction site would be necessary. The latter does not mean that the USSR, after years of refusing on-site inspection, has decided to allow them after all; but the report reflects agreement on more details of on-site inspection, and in this sense it may indicate a slight softening of the Soviet attitude.

What did emerge, however, from a visit the group made to the Swedish pharmaceuticals factory KabiVitrum AB, is that that on-site inspection need not jeopardise commercial secrets. In recent years, the Workshop has visited

commercial chemical factories in West Germany and the United States, and a US Army chemical/munitions disposal facility.

Next year, the group plans to visit a site in East Germany. The visits have enabled the group to explore verification questions which could arise under a CW convention. Invasion of chemical factories by inspectors has long been opposed on grounds of commercial privacy; but as the Pugwash group visits more facilities in more countries, its experience suggests that opposition to some forms of international control of the chemical industry rests on shaky ground.

It was also reported that the discussion on confidence-building measures (CBM's), provoked more reaction than usual. CBM's are steps the sides can take to increase mutual trust: voluntary exchanges of technical and methodological information on chemical research and development related to CW problems, for example. The more trust there is between the sides, the fewer formal verification procedures are needed. Various CBM's have been discussed by previous meetings of the Workshop, but this one produced an additional concept for increasing mutual trust: 'obligatory CBM's under a CW convention'. The group felt that it could be useful to treat certain sorts of treaty provisions as CBM's: provisions, for example, which might be valuable but difficult to verify under a treaty. The discussion on CBM's was laced with controversy about which measures would be best to adopt; and at least one participant was surprised that both sides agreed to mention them favourably in the report.

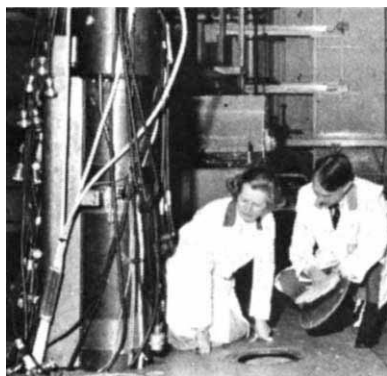
Wendy Barnaby

news in brief

Gluons at DESY: Results from the PETRA electron-positron storage rings at DESY, Hamburg, are being taken as confirmation of the existence of the 'gluon'. This massless particle, is according to 'quantum chromodynamics', the mediator of the strong force between quarks: it is the 'glue' which keeps quarks bound into particles such as the proton, neutron, and other hadrons. Quantum chromodynamics (QCD) has been developed in analogy to quantum electrodynamics (QED), the immensely successful relativistic quantum theory of electromagnetism. The gluon in QCD plays the role of the photon in QED. However its discovery may not foretell the 'technological revolution' seen by the *International Herald Tribune* on 30 August: like the quark, it has proved impossible to separate from the hadrons of which it forms a part. The experimental evidence for it, now gathered by several teams at DESY, is a set of events showing the production of particles correlated into three 'jets' or streams — a signature of the decay of a gluon (see *News and Views*, page 104). Early evidence for the gluon was presented by one of the teams at CERN, Geneva, in June; this has been corroborated by data presented at Fermilab, Chicago, by a collaboration headed by Samuel C.C. Ting.

Britain's nuclear future:

Britain's Prime Minister, Margaret Thatcher (right), last week reaffirmed her intention to press forward with a nuclear programme for Britain. After starting-up the new fuel reprocessing plant in Dounreay, Northern Scotland, she said that personally she would like to see the go-ahead for a full sized commercial demonstration fast reactor possibly at Dounreay, but there would be a public inquiry first. She expected this decision to be made within two years, and then another eight to ten years to pass before the first commercial station was built.



Britain will also soon have its first pressurised water reactor. The Central Electricity Generating Board has been looking at four PWR designs and is expected to recommend the US Westinghouse design. Sizewell in Suffolk, Dungeness in Kent and Hinkley Point in Somerset are all possible sites currently being investigated by the CEGB.

Meanwhile Friends of the Earth with other anti-nuclear bodies have been discussing ways of improving their effectiveness in the anti-nuclear campaign. According to Czech Conroy from Friends of the Earth, they want a "better coordination of our opposition to nuclear power".

ICRP lung models out: UK National Radiological Protection Board studies published in the Board's latest annual research and development report have shown that industrially produced radioactive dusts do not necessarily behave as predicted by the International Commission on Radiological Protection models. Very small particles of plutonium-239 dioxide, about 0.001 microns in diameter, have been found to be soluble in blood and move rapidly through the body, meaning the radiation dosage to the liver and bone could be up to ten times greater than estimated by the ICRP models. Normally only 0.1% of industrially produced plutonium dust is soluble, but if the plutonium aerosol is produced in conjunction with sodium, for example in fast breeders where sodium is used as a coolant, up to 50% of the aerosol is composed of these small, soluble particles. However, the NRPB studies have also shown that the amount of plutonium

entering the blood and deposited in extrapulmonary tissues following inhalation of actinide dusts over 1 micron in diameter, have been overestimated by a factor of ten by the ICRP.

Scientists attack Carter's approval of new missile system: The Federation of American Scientists has strongly criticised President Carter's decision, announced at the end of last week, to proceed with the deployment of a new generation of intercontinental ballistic missiles, the MX. After several years of studying alternative proposals for the design of the new system, the President has selected one in which 200 missiles will be deployed in underground shelters, mounted on lorries which can move them between alternative launch sites sufficiently fast to avoid a Soviet missile attack.

The whole deployment programme is expected to cost 32 billion dollars. In its statement, the FAS claimed that the MX "is not just a mistake but a disaster". The federation also voiced fears that the deployment of MX missiles would not only lead to escalation of the arms race, but would also render the Strategic Arms Limitations Treaty untenable, and might result in the abrogation of the anti-ballistic missile treaty. The statement questions the strategic value of the MX — and predicts that it will never be built.

Hungary and UK revise science and technology agreement:

Hungary and the United Kingdom have drawn up a new programme for long-term cooperation in applied science and technology. In practical terms, this simply continues existing cooperation projects with one or two additions such as metallurgy and communications engineering. Formally, however, it represents a compromise between the Hungarian and British approaches to such exchanges.

The original UK/Hungarian agreement for cooperation in science and technology was signed in 1967 under the umbrella of the Intergovernmental Joint Commission, which covers all aspects of economic relations between the two countries. In 1977, however, the Hungarians wanted to take scientific cooperation out of the sphere of the Joint Commission, and draw up a special agreement. Exchange agreements between Western and Comecon countries have tended to link trade and scientific exchange probably because scientific cooperation is handled on the British side by the Department of Trade, and on the Hungarian side by a mixed panel representing the relevant ministries. A special agreement could have rationalised this structure, but this very difference made it impracticable to give science and technology an agreement of its own. It does, however, now have its programme formulated in a separate document.

Argentina accused of responsibility for deaths and torture of scientists:

The government of Argentina bears "ultimate responsibility" for permitting the disappearances, tortures and deaths of many Argentine scientists, according to a statement issued in Washington last week by the National Academy of Sciences and the American Association for the Advancement of Science. It says that the Argentine government has been "totally unresponsive" to repeated inquiries made into the fates of many scientists, engineers and students who have disappeared since the military government seized power in March 1976.

Such inquiries had been made by both individuals and groups within the scientific community, including the American Physical Society, the American Psychological Association and the Massachusetts Institute of Technology. "The problem is not solved by the government's assertion that it knows nothing of the fate of these 'disappeared' citizens or that they are victims of terrorists," the statement says.

It adds that Argentina cannot have a respected place in the world of international science until the respect for human rights of all citizens permits honest inquiry and freedom of discussion without fear for personal safety.

Why France has had no public nuclear debate

France has not had the public debates on science and technology of other western nations. **Pierre Papon**, of the Ecole Supérieure de Physique et Chimie Industrielles, Paris, outlines why.

ALTHOUGH the past five years have seen considerable — and sometimes violent — political debate in many western countries over the objectives and implications of technological and scientific advances, notably nuclear power and genetic manipulation, France has been remarkably free from public discussion or dissent. The French have traditionally left decisions on science policy to the government in power, and nowhere is this more evident than in the nuclear power programme. Despite its wide socio-political implications, the main decisions on nuclear power have been taken solely by the government, along with a governmental agency — the Commissariat à l'Energie Atomique (CEA) — and the public utility, Electricité de France.

Political decisions have been taken on at least three important occasions in the history of the French nuclear programme. First there was General de Gaulle's decision, based on advice from R. Dauty and the physicist F. Joliot, to found the CEA in October 1945. After the scientific and technological infrastructures had been built up, the parliamentary vote in July 1952 for the first five-year plan for the CEA was a new decisive step. This plan included the building of thermal reactors producing plutonium, which were to form the basis of the future military nuclear programme, launched under the initiative of the then Chief Administrator of the CEA, P. Guillaumat. Although it began slowly, the military nuclear programme accelerated after 1958, and the government eventually decided to shift from gas-cooled reactors to pressurised water reactors. This major decision in 1969 was endorsed five years later when the Messmer government embarked on a new nuclear programme.

Under this plan, the EDF was authorised to build and operate a nuclear capacity representing 38GW of installed power, supplying 50% of electricity demand in 1985. This will include 900MW PWR nuclear reactors, and more powerful 1300MW reactors built by Framatome under licence from Westinghouse. The programme will be completed by breeders, the first commercial prototype being the 1200MW Superphenix reactor which is being built at Creys-Malville, Isère, by an industrial society in cooperation with Italy and West Germany. The French nuclear programme is thus extremely important, despite delays and high costs.

Scientists' reactions

After the government had decided to launch this large nuclear programme in

1975, a group of some 400 scientists reacted against the magnitude of the programme and the lack of public debate on nuclear problems. As the 'Groupement de Scientifiques pour l'Information sur l'Energie Nucleaire' (GSIEN) these scientists, mainly physicists, publish regular newsletters giving the main scientific and technical data relevant to the nuclear debate and expounding the socio-political implications. Since it has political contacts and an honest audience, this group has performed a useful task in disseminating information. The union of workers of the CEA, affiliated with the CFDT, has regularly warned the authorities of the technical and social risks of the nuclear programme; recently for example, they drew attention to safety problems related to working conditions at the fuel reprocessing plant in La Hague. The action of the physicists in promoting a public debate on nuclear problems, however, has no equivalent in other fields: a group of biologists, among them a few connected with Pasteur Institute, met with little success when they tried to initiate a public debate on recombinant DNA research a few years ago.

The Three Mile Island accident has recently put increased emphasis on the question of nuclear reactor safety. The French Physical Society (Société Française de Physique, SFP) the professional society of physicists, seized this opportunity for opening a debate on the problems of reactor safety at its biennial general meeting in Toulouse in the last week of June. The discussion now conducted by a panel of physicists, which included two members of the CEA (M. Tanguy, Head of the Protection and Nuclear Safety Institute and M. Droulers, Director of the Department of Evaluation of Safety of Reactors) and was chaired by B. Dreyfus, vice-president of the society. The members of the CEA were questioned on the Harrisburg accident and on its implications for the French nuclear programme, especially for the safety of the PWR reactors. The debate focused mainly on technical and scientific problems, such as the behaviour of the primary coolant in biphasic conditions, the crucial problem of instrumentation inside the containment vessel, the reliability of data available to the operators, and the probability of major accidents like those occurring after a thermal shock or a core melting. Questions were also asked about the technical competence of reactor operators and their ability to cope with a major accident.

The participants also tackled the question of whether outside scientists should be involved in planning and executing nuclear safety research programmes. The consensus was in favour, but no precise agreement was reached on how such participation could be achieved. The CEA physicists did display confidence in the PWR reactors, although they admitted that some events which occurred at Three Mile Island had not been taken into account in considering accident risks, including the formation of a large hydrogen bubble, and they also conceded that more attention should be paid to the behaviour of the secondary system and to the instrumentation of the primary coolant.

Although this meeting is not a landmark in the nuclear debate, it is important in that it constitutes the first attempt by the Société Française de Physique to open a discussion among its members on an important element of the nuclear debate. Other discussions, on the fuel cycle and on various reactor systems (including breeders), are planned.

Government traditions

The reasons for the lack of previous public debates on major technological issues like nuclear power lie largely in the traditions of French government. Until the second world war, successive governments held science and technology to be low priorities in national policy, although they kept a tight hold on some industrial areas like energy supply. With the post-war move into high technology, state intervention took place in research areas important to France's technological independence, beginning with nuclear power in 1945, and extending to aerospace and computers in the 1960s.

Because of their national strategic importance, decisions in these fields have been felt to be the responsibility of governmental decree rather than laws passed by parliament.

In the nuclear power field, there have been a few debates in parliament, and no general legislation has been passed. For example, although the breeder Superphenix is partly financed through the budget, the decision to build such a reactor was taken by a decree in May 1977 without a vote in parliament. France does not have any procedure of public inquiries to allow the assessment of major technological programmes with the participation of independent experts, as in the Windscale inquiry in the UK. These two facts have combined to weaken the possibility of a serious political debate.

Parliament's role is a rather weak one, since it has no standing committee on science and technology as such. The 'Commission des Finances' deals with major problems of science and technology in the National Assembly, but these two committees do not have sufficient technical means to investigate complex technical problems. In April 1979 a law creating a

special permanent committee for the assessment of major technical proposals was passed by the National Assembly — against the opposition of the Minister for Industry A. Giraud (a former head of the CEA), who declared it unconstitutional; it is now before the Senate.

After the Harrisburg accident a special investigation committee was set up by the National Assembly and its four members went to the US. In their preliminary report, published on 27 June, the deputies insisted on the necessity of informing the public about nuclear safety, and they insisted that the government should publish the intervention plans prepared in the case of an accident. This report, although useful, falls short of the full-scale assessment needed of the safety problems involved in the nuclear programme.

In France it is also traditional that the government resists efforts to make public all the factors on which its decisions are based. In the energy domain the weight of the powerful public organisations EDF and CEA is not balanced by other institutions challenging their conclusions.

The high-ranking Civil Service considers that it has, as the ultimate political power in the country, the right to intervene in high technology sectors and to control the decision-making process. Information also comes under its power, and it is reluctant to abandon even some of this to parliament, which has indeed rarely shown a real interest in the problems of science and technology. The creation of a special inter-ministerial committee, chaired by Mrs Simone Veil, former Minister of Health, which will disseminate information on nuclear energy is a step in the right direction. Unfortunately this committee has devoted most of its time, since its creation more than a year ago, to simply informing itself.

Another element of the problem is that the French government usually trusts what L. Kowarski called 'les compétences instituées' (representatives of public institutions, business, large organisations, and so on). It is typical that the government asked a panel of members of the Académie des Sciences to comment on the official report of the Harrisburg accident; and, given the reluctance of the Assembly to engage in controversial matters, one can estimate how useful their opinion will be.

Competent experts who may have views and opinions which conflict with those of the government are not involved in the decision-making process. To be fair one must add that the scientific community, with few exceptions, has rather fought shy of all problems related to the implementation of science and technology. It has tended to expound its views on the general situation of research, and retreated from questions with social or political overtones. One may hope that the severity of the social and economic crisis will now oblige the government and the scientific community to enter a dialogue with public opinion. □

French scientists oppose CNRS reforms

A STORM is building up within the French scientific community around the government's proposed reform of the country's highly centralised research organisation, the Centre National de la Recherche Scientifique (CNRS). In August nearly 200 scientists, engineers and technicians demonstrated against the plan — and this in a month when nearly all the French go on holiday. In addition, a lengthy polemic has been opened in the national press on the subject while the main trade unions involved in the CNRS are promising further activity this month.

The reforms, proposed by Giscard d'Estaing's government, have two basic themes: a structural reorganisation of the CNRS itself and a change in the direction and control of research goals. The remit of the CNRS runs from pure mathematics through physics, chemistry, biology to linguistics, musicology, history and philosophy. Thus any change is bound to have a major impact. It was originally set up to develop the career of "research scientist" independent of university teaching and as a profession in its own right. Currently its budget is FF3,500 million (more than £35 million); it employs 8,300 research scientists (more than one half the national total) and 13,000 engineers, technicians and clerical staff, all its employees enjoying the rank of civil servants.

The CNRS is presently governed by a rather Byzantine array of interlocking committees and directorships. The original plan envisaged a system with most committees at the top having both elected and appointed members. Thus, for example, the National Committee for Scientific Research, which is supposed to oversee part of the budget and the performance of the various laboratories, is composed of 41 subcommittees, each covering a separate discipline, and each with 26 members — 15 elected by the staff and 11 named by the government.

In fact another more hierarchical structure has grown up within the CNRS. It consists of seven directors, each in charge of a commission which oversees the work of all labs in its area. The directors report to the director-general of the CNRS, currently M Robert Chabbal. There are no elected members in this chain which has now established *de facto* control of finances and the review of the work of the centre. In the words of M Chabbal, "the French like pyramidal structures."

The first part of the proposed reforms will "regularise" this unofficial structure, giving it permanent form and official control. Although the National Committee would continue to exist it would have only advisory powers, while real power will lie with the appointed directors and their commissions. The main thrust of this part of the reform then is to introduce a more "streamlined" though less "democratic"

management with more autonomy for the different fields of research.

The three unions, who represent a substantial number of the scientists, engineers and technicians at the CNRS, and whose members took part in the August demonstration, are calling for militant action against the reform. Meanwhile a petition circulating among researchers at the CNRS had gathered 230 signatures within the first fortnight of its launch.

The second aspect concerns the structure of research itself. The traditional "vertical" division of the CNRS by discipline has for some time been supplemented by "horizontal" intra- or inter-disciplinary projects. Examples of these are the 30 or so GRECO (Groupements de Recherches Coordonnées) agreements which reflect national level single discipline studies and the more than 40 GIS (Groupements d'Interets Scientifiques) or cross-disciplinary studies, for example, the CNRS/University of Montpellier/local authorities solar energy project. The reform calls for an increased emphasis on these "horizontal" structures with more input and responsibility being given to the CNRS's partners, particularly industry.

What is feared is that the real aim of this reform is to dismantle the centralised structure of the CNRS with forcible merging of laboratories, "nationalisation" of the work force and the placing of the resulting smaller and more vulnerable pieces under private industry. In particular, the social and human sciences are scarcely visible in the new scheme while the more "physical" disciplines will be heavily weighted towards rapid and easy profitability.

On the one hand, the director-general M Chabbal, stresses the advantages of more fluid discipline boundaries and increased ease of formation of new research areas. On the other, opposition scientists and unions warn of private industry's control of research goals and the loss of autonomy of the scientific community. Whether a major confrontation will actually develop depends largely on the government's tactics. In France, it is not uncommon for proposed legislation to be postponed even after its announcement or for bills, once passed, to be left unenforced.

If the reforms go ahead one result may be the opening up of debate on the nature of research itself, which is beginning to be discussed in the socialist trade union CFDT. Are scientists, private industry and the State the only possible judges of the value and direction of research? What about the people themselves and their participation in research decision?

Jim Ritter

Jim Ritter is in the Department of Mathematics, King's College, London.

Nuclear India

This week a law comes into operation in the US which will have the effect of foreclosing nuclear fuel supplies to India.

Anil Agarwal here considers India's nuclear future

THERE is no more emotive tissue in the field of science and technology in India today than atomic energy. Since the 1974 nuclear test at Pokhran, the Indian Government has been under enormous pressure from western nuclear powers — in particular US and UK — to sign the Non-Proliferation Treaty (NPT). India has always insisted that the Pokhran test was entirely intended for peaceful purposes and that the NPT is an extremely discriminatory document, which does nothing to curtail the proliferation of nuclear weapons in the existing nuclear weapon states.

While all Indian governments had regularly denied any interest in nuclear weapons, Prime Minister Morarji Desai turned the making of any nuclear bomb by India into an even stronger moral issue than had his predecessors. In characteristic style, Mr Desai told western reporters after the Commonwealth meeting in London in 1977: "I'll give it to you in writing that we will not manufacture nuclear weapons. Even if the whole world arms itself with the atom we will not do so". Recently, when the news broke that Pakistan was involved in building a uranium enrichment plant, there were demands from several members of parliament that India should make its own nuclear weapons. But Mr Desai again stated: "I am neither prepared for the manufacture of the atom bomb nor will I sanction funds for it".

When Mr Desai was Prime Minister, he closed Indian options for peaceful nuclear explosions, which all earlier Prime Ministers had studiously kept open. He even criticised the Pokhran explosion, which had been proclaimed a major national achievement during Mrs Gandhi's term in office — a view shared by most of the country's intelligentsia.

Mr Desai told parliament that Mrs Gandhi had conducted the Pokhran explosion for political rather than scientific gains. Mr Desai immediately faced a barrage of criticism. "Your unilateral declaration abjuring nuclear explosions even for peaceful purposes and ambiguous and contradictory statements on nuclear policy have raised profound misgivings about the future scope and objective of our nuclear programme," wrote an angry Mr Y.B. Chavan, in a letter to Desai in late 1978. Mr Chavan, a veteran Indian politician, who was then leader of the Opposition and is now Deputy Prime Minister in Mr Charan Singh's government, added: "we were very surprised and disturbed by your extraordinary statement on the Pokhran



India's underground nuclear test in Rajasthan: Desai condemned it

explosion. You may have had your own reasons for making it, but in the process you chose to pronounce on the technical aspects of the explosion, denigrating our scientists and engineers who were involved in the project. It has been recognised by experts all over the world that technically the Pokhran explosion was a fine job.

Mr Desai replied reiterating his stand. "It is quite clear to me" he wrote, "that you do not know the details and the lessons that were learnt. Technically there is no doubt that it was a good effort but compared to achievements elsewhere it was a very small effort." Moreover, the explosion had created international problems for India. He explained that the "explosion is always held against us as a preparation for warlike use of atomic energy and you know very well how it is being exploited by our neighbours so that they can blackmail foreign governments into assisting them in acquiring that capability themselves."

With foreign nuclear powers, Mr Desai took a straightforward moral position. He agreed to throw open all the nuclear reactors in India to international inspection and accept fullscale safeguards, but only on the condition that the nuclear powers themselves sign a comprehensive nuclear test ban treaty, agree to reduce their own stockpile and open their own reactors to international inspection. Mr Desai informed Mr Chavan in his letter: "In my private discussions both with President Brezhnev and President Carter I have even made it clear that unless the safeguards are extended to military reactors they cannot insist on our having them."

When Mr James Callaghan, the then UK Prime Minister, followed President Carter to negotiate with Mr Desai, just a week after the latter's visit to New Delhi in early 1978, Mr Desai once again refused to sign the NPT. But he agreed to open India's reactors to international inspection once there was a basic international agreement amongst UK, USSR and USA for a comprehensive test ban treaty. By leaving out France and, more important, China, which

is the immediate nuclear power on India's borders, even several western observers accepted that Mr Desai had made a significant concession. Earlier this year, President Carter publicly admitted that he found it difficult to argue with Mr Desai's moral position.

However, within India itself Mr Desai was strongly criticised by almost all leading newspapers and politicians for his soft and yielding stand over NPT. Many commentators said that he had set India on a slow march towards acceptance of international safeguards. A leading columnist of the Times of India, wrote: "Mr Desai is a man of high principles. He is a conscientious objector to nuclear weapons under any circumstance. It is to his credit that he would rather resign than do anything against his conscience. Even so, must he forswear, on behalf of India, nuclear weapons for all time to come, regardless of the circumstances? As a senior member of (former Prime Minister) Mr Nehru's cabinet, he must know that it was with the full concurrence of the then Parliament that Panditji had declared himself to be opposed to going in for nuclear weapons. But he had taken care to add that though he hoped that future parliaments would agree with his view, he would not bind them down. This is a more rational approach than a blanket rejection of nuclear weapons. And the least that the Prime Minister ought to do is to seek the present parliament's endorsement for his stand."

When the Indian government agreed to a US proposal to set up an independent committee of nuclear experts to study, as Dr Joseph Nye of the US put it, "the question of what safeguards options were available for states which were not members of the NPT", there was immediate suspicion amongst politicians and press commentators that the country may have walked into a trap. The reasons for setting up the committee have never been sufficiently explained but it seems US diplomats had claimed that the committee's deliberations would help to soften public opinion in the US against the supply of nuclear fuel to India.

However, until the western powers made up their minds about a comprehensive test ban treaty, Mr Desai's government took careful steps to ensure that India does not have to accept fullscale safeguards on its reactors. The constant irritant in Indo-US nuclear relations is the Tarapur Atomic Power Station. The US government is obliged under a 1963 agreement to supply enriched uranium fuel for the entire life-time of the station. But under a new law, it now has to stop supplying nuclear fuel to any country which has not signed the NPT by March 1980. The law does not even exempt sales to those countries with whom the US had previous agreements. Naturally, the Indian government has protested against it.

Over the past few years there have been extraordinarily long delays in supplying nuclear fuel to India. The last consignment of fuel arrived in March, 1979, about 13 months after the Indian government had applied for it. At that time, the US government had not even presented to the Nuclear Regulatory Commission, which permits sales of nuclear fuel, another Indian application sent in September 1978 for yet another outstanding consignment. Many Indians felt that the March consignment too had been sanctioned only because Indian leaders had started discussions with the USSR over the supply of enriched uranium. But because of these delays, the Nuclear Fuel Complex at Hyderabad, which manufactures fuel elements, had to close down its workshops. The new US law forbids the government from considering further applications for nuclear fuel sales after 10 September.

The US government had believed that the new law would force the Indian government to see its viewpoint. But Mr Desai, strongly supported by Indian public opinion, informed the US government that refusal to supply enriched uranium for the Tarapur reactors would be treated as a unilateral abrogation of the previous agreement. India would have no further obligations to observe the bilateral safeguards which are imposed on the reactors at the moment and would be free to reprocess the spent fuel from them. Under the existing agreement, the US has the right to buy back the fuel.

Plans to use plutonium

No decision about the spent fuel has yet been taken and it is being stored in reservoirs which are now full. The storage capacity is to be expanded, but as soon as the legal position about the fuel is clear, India would like to extract plutonium from it at its waste processing plant at Tarapur. The Indian Atomic Energy Commission claims to have developed a new fuel consisting of natural uranium enriched with plutonium to replace the enriched uranium fuel supplied to the US.

Scientists at the Bhabha Atomic Research Centre have indigenously designed and built a plutonium-fuelled

small experimental reactor so India has experience of working with plutonium fuels. With fuel supplies that have already been agreed upon, the Tarapur station should be able to generate power until 1982. That should give the Atomic Energy Commission time to fabricate the new fuel.

The option of using mixed plutonium and uranium oxide fuels could embarrass the US government which has always been against the use of plutonium in thermal reactors, and has even tried to prevent European countries from using it. Its diplomatic offensive against India might well produce a situation it has otherwise tried to prevent. Once plutonium starts being used in thermal reactors, the total amount in the world could jump from 25 tonnes to well over a thousand tonnes, making it almost impossible to police its use. The fuel supply for Tarapur is, therefore, supposed to be an open issue. The US President may well decide to continue to supply nuclear fuel to India to stop this precedent from taking place. There is, of course, the even more important worry that Indo-US relations would take a nose-dive.

The Desai government had also taken steps to prevent fresh safeguards being applied to the Rajasthan Atomic Power Station (RAPS). After the Canadian government had unilaterally broken its agreement with India in 1976 to supply heavy water for the second unit of RAPS, Mrs Gandhi's government had started negotiations with the USSR. The Russians, however, insisted on even stronger multiple-point safeguards than the Canadians. An agreement with the USSR was concluded in 1977, soon after Mr Desai's government took office. Under it the IAEA has the right to inspect any other nuclear system in which any material generated in the Rajasthan station is used. India has an experimental fast breeder reactor under construction at Kalpakkam, where its second major nuclear research centre is being developed. RAPS-II is now reported to be ready but the Russian heavy water has not yet been used. Until it is, the safeguards agreement does not come into operation. It is possible that India will keep the RAPS-II idle until it can manufacture its own heavy water.

Apart from the Tarapur station, all the Indian stations are heavy water-moderated, natural uranium-fuelled, CANDU-type reactors. India manufactures its own natural uranium fuel, but remains critically short in its heavy water requirements, and is thus dependent on foreign sources. Apart from a small heavy-water production unit in operation at Nangal, there are four large plants under construction. The first is being built by Indian engineers at Kota. Designs for this plant were obtained from Canada but even the Canadians have found difficulties with similar plants. The Kota plant too has been plagued with difficulties and delays, and

has thus seriously undermined India's plans to gain expertise in heavy water production. It is expected to begin operation in 1980.

Three other plants are being built at Baroda, Talcher and Tuticorin by French and West German companies. All are suffering delay. The Baroda plant started trial runs several years ago but a chemical explosion seriously damaged it and it is now being repaired. The Talcher plant was delayed when two massive steel towers which had been shipped from Europe got mysteriously swept away at sea. Both the Baroda and Talcher plants are now expected to start operating by the end of this year. The Tuticorin plant has been undergoing trial runs but it has been plagued with problems in the ammonia plant of the fertiliser factory with which it is linked. Once these plants begin operating, they should be able to supply the heavy water needed for the nuclear reactors under construction.

The Indian nuclear power programme itself has been lagging behind the schedules projected in the early 1970s because of serious management and construction problems. Nuclear stations at Madras and Narora, which are under construction, are expected to take more than ten years to build, and their costs have escalated considerably. This has led to resistance from both the Ministry of Energy and the Planning Commission to sanction funds for more nuclear reactors. This situation existed under Mrs Gandhi's government but Mr Desai told parliament that the government wanted first to cope with the atomic power plants already taken up before thinking of setting up new ones. A fifth plant has been under consideration for several years — either in the State of Gujarat or as an extension to the existing Tarapur Station. Mr H.N.Sethna, Chairman of the Atomic Energy Commission, claimed a few weeks ago that its location may be decided by December.

The slow progress of the nuclear programme has certainly left young engineers working with the Atomic Energy Commission in a very uncomfortable situation as far as their careers are concerned. The plans formulated in the early 1970s had envisaged that one nuclear power station would be sanctioned every year. But now many senior engineers have remained stuck in their old positions for over a decade as they have no new sites to move to, and their junior colleagues have remained in their junior positions. It is the same story, for different reasons as in many developed nations. But the under-employment of engineering talent — some 30,000 working in India atomic energy establishments — is a much more severe problem in a developing country. The unfortunate thing is that as long as external foreign pressures exist, no government in India will find it easy to evaluate seriously the role of nuclear energy in its future energy development programmes. □

correspondence

Biased reporting of East European science

SIR, — There should be little doubt nowadays that science needs extensive, systematic and friendly international ties for its own progress, for positive service to humanity and for contributions that such ties make toward safeguarding peace and avoiding the world-wide devastation which advanced science and technology have guaranteed to be inherent in a war between major powers. Even arms limitation and, hopefully, arms reduction are not only general human concerns but also professional issues affecting the direction of science and the question as to whether its major efforts will be oriented toward destruction or toward human welfare.

The near catastrophe this year at the nuclear plant at Three Mile Island, Pennsylvania, has underscored dangers which could arise even in the absence of war, and leads one to be grateful for the existence, since 1973, of the US-USSR Joint Committee on Co-operation in the Peaceful Uses of Atomic Energy. Its fifth meeting (Washington, April 1978) gave prominent attention to work on 'Light-Water Reactor Safety' — the type of reactor used in the US — and to plans for intensifying work on this topic, whose importance the whole world now appreciates.

A sense of responsibility to science, to peace, to mankind would require the encouragement of such co-operation and its substantial expansion.

It is, therefore, very sad to observe *Nature's* failure to exercise this responsibility, and its degeneration into a propaganda sheet for those anxious to whip up an hysterical ill-will against East European science. In what has assumed the dimensions of a campaign, it seems to be doing what it can to encourage those seeking to destroy co-operation, or even contact, between the scientific communities of Eastern Europe and those of the US and its allies. News articles do not conceal their bias and editorialising. For example, a news report (7 September 1978, page 3) under the headline 'Boycott of Soviet contacts is for individuals, says NAS [National Academy of Sciences]' stated early on "This reaction would appear to substantiate the case for boycott, a case most ably put by Valentin Turchin". The rest of this 'report' is similarly tendentious and argumentative. Incidentally, the Turchin letter and similar ones were published, not in the space normally reserved for letters, but more prominently, in larger type and with conspicuous headlines.

The author of that article is a regular contributor to *Nature*, Vera Rich. Her numerous articles are chiefly propagandistic, lacking in analysis, highly selective, crowded with sneers and sarcasm, ill-mannered and disruptive, and contemptuous of Soviet science. They do not offer the reader any well-rounded picture of East European scientific life and developments, to say nothing of the fruits of international scientific cooperation.

Underlying all the sarcasm and the jibes is the unwarranted assumption that Soviet science and the science of other socialist countries is very much the junior partner in any cooperation. Occasionally this is stated explicitly, and such views given more or less unchallenged publicity. One anonymous letter (14 December 1978) asserts that in 1965 the US and the USSR had about the same number of scientists, but that the former did one-third of world science, the latter only one-sixth. Leaving aside measurement

problems, one might ask why *Nature* didn't point out that through its money the US has fortified its science establishment by visiting upon other countries a 'brain drain' which has caused even its closest allies to complain and which has damaged developing countries, while the USSR has relied almost exclusively on its own population. As a perhaps atypical example, it might be noted that the School of Mathematics of the Institute of Advanced Study at Princeton has normally been staffed at the professional level by a majority of great imports.

In any case, Soviet and other socialist scientists are no 'junior partners' in international science, no matter how *Nature* portrays them.

But the blame for this destructive campaign cannot be placed on Vera Rich's pen alone. *Nature*, itself, has weapons in its arsenal. Some day a doctoral dissertation will analyse headlines, positioning, type-sizes, linguistic techniques and much else, to the amusement of the author and the embarrassment of the editor.

Without awaiting such refinements, I am concerned about such things as an anonymous full-page article (13 October 1977, page 548) by what *Nature* calls "a correspondent with some experience of East European countries, . . . [who] returned [from the GDR] with these impressions". No explanation is given as to why this correspondent was permitted a cloak of anonymity to shelter an ungracious guest's snide barrage. *Nature's* editorial introduction to this piece declared "Scientific exchanges with the German Democratic Republic (GDR) are rare, and our knowledge of the state of its science is consequently sparse". It neglected to mention that NATO countries refused until recently to recognise the GDR, excluded it from the UN, and were unwilling to negotiate exchanges earlier, or even to permit GDR citizens to visit under their own passports. Every effort was made by NATO to isolate the GDR. For years the International Mathematical Union allowed its FRG-affiliate to act for both German States.

In passing, let me mention that I have made three brief scientific visits to the GDR (in 1965, 1972 and 1978), and that the colleagues I met there were well-trained, skillful, enthusiastic scientific workers who were far from conveying the "frustration and pessimism" which your anonymous correspondent claimed to be the prevailing mood.

I hope that the editors will not await future doctoral candidates to analyse the myriad ways in which *Nature* demeans itself today. *Nature* is an important voice in the international scientific community. Its editors should take cognizance of the grave responsibility this implies, and should bestir themselves to establish some objectivity, balance and reason into its activities impinging on the crucial field of scientific co-operation between the countries of Eastern Europe and other major scientific centres.

Yours faithfully,

LEE LORCH

York University, Ontario, Canada.

Uranium use in fast reactors

SIR — R.D. Smith in his letter on fast reactor safety (23 August, page 630) says, "remember the fast reactor will produce about 60 times more energy than a thermal reactor for the same quantity of uranium".

I thought this particular piece of nuclear

nonsense had been scotched for good. It might be true in 200 years time, but certainly is not for any period which is significant for our present situation. When challenged by Leslie Granger and David Merrick (*Energy Policy* December 1976, and *Nature* 16 December 1976, pages 596-8) Bart Cutts, Head of Technical Services at UKAEA eventually (*Energy Policy* September 1977) presented a "notional programme of electrical installation". One is justified in assuming that it was the best case he could produce, especially as he found it necessary to assume a very low growth rate for electrical energy of 2½% p.a. Even so the ratio of utilisation of uranium for a programme based on the maximum development of FBRs and one based only on thermal reactors was 1.1 by 2000 and only 1.7 by 2020, compared with this absurd figure of 60 times which might be achieved by 2200.

For the sort of increases in electricity production that used to be achieved and were projected at least until 2000, i.e. anything over 5½% p.a., Granger and Merrick have shown that the ratio cannot exceed 1½ times as long as this rate of increase continues. The reason is simply that, as Walter Marshall has said, fast breeder reactors are really breeder fast reactors, or perhaps better, slow breeder fast reactors. To achieve even the rate of increase, modest by historical standards, of 5½% p.a. it is necessary to build large number of thermal reactors to provide most of the increase and furnish plutonium to start up the FBR series. These gradually breed more plutonium, and the process goes asymptotically to the use of 1½% of the uranium instead of the 1% for thermal reactors. The higher the annual increase is above 5½%, the more slowly is this figure of 1.5 approached, never to reach any higher unless the annual increase comes below 5½%. As we have seen, even for an annual increase as low as 2½% p.a. it only goes to 1.7 by 2020.

On a related point, in order to reach a stage when further supplies of uranium are no longer required, the rate must be below 3% p.a. Above that the need to import uranium never ceases.

It is to be hoped that R.D. Smith's corrections of Norman Dombey (other than the misprint) are rather more relevant than his reference to the improved utilisation of uranium.

Yours faithfully,

J.W. JEFFERY

Birbeck College, University of London, UK.

Defending science and rationality

SIR, — I have just read your editorial (23 August, page 619), on 'Science, nonsense and responsibility'. I do so with that slightly sour amusement always induced by the spectacle of Satan denouncing sin. Having read your editorial and review columns for many years, I find your pose as a defender of science and rationality decidedly unconvincing. However, if you should now switch to opposing the major as well as the trivial superstitious movements of our time, no one would be more pleased than I.

Yours faithfully,

M. HAMMERTON

University of Newcastle upon Tyne, UK

news and views

Structure and force generation in muscle

from John M. Squire

THE sliding filament model of muscular contraction, proposed 25 years ago, is now generally accepted to account for the shortening in muscle; the thick myosin filaments interdigitate with the thin actin filaments and when these filaments slide past each other the muscle changes length. However, within this basic scheme, relatively little is known about the structural details of the force-producing mechanism which causes the filaments to slide. The thick filaments contain projections (the myosin heads or crossbridges) which can split ATP, which can bind to the neighbouring actin filaments and which have an ATP turnover rate that is increased by actin binding. Tension is related to ATP usage, so the crossbridges are clearly implicated in force generation. It is commonly assumed that the crossbridges in an activated muscle first attach to actin and then change their attachment configuration through a number of mechanical steps during which force is generated. The crossbridges subsequently detach from actin and reset themselves ready for a further attachment cycle. Although the kinetics of the biochemical steps in the actomyosin ATPase cycle are fairly well defined (see Lynn A. Rev. *Biophys. Bioeng.* 8,145; 1979) the details of the corresponding changes in structure are still relatively uncertain and much effort is being devoted by structuralists to defining the sequence of mechanical steps involved in force generation.

One of the most useful experimental probes available to the structuralist is the X-ray diffraction technique, since, unlike electron microscopy, it can be applied to muscle which is living and, if required, actively producing tension. Of the various regions of the resulting X-ray diffraction patterns which can be studied, one of the most informative is the equator. This is the region which provides information about the density profile of the muscle when viewed down the muscle axis. Figure 1 shows schematically the appearance of such a view in the A-band of vertebrate skeletal muscle. Here the myosin filaments (M) form a hexagonal array with the actin filaments (A) at the trigonal points of the lattice. The principal diffracting planes in this lattice are the $10\bar{1}$ and $11\bar{2}$ planes as indicated in Fig. 1. The actin filaments lie

between the myosin filaments when the assembly is viewed along the $10\bar{1}$ planes, but are in line with the myosin filaments in a view along the $11\bar{2}$ planes. This means that if there is a movement of mass from near the myosin filaments to near the actin filaments when a muscle is activated, the effects on the $10\bar{1}$ and $11\bar{2}$ X-ray reflections will be different. More mass at the actin filaments will tend to smooth out the density profile in a view down the $10\bar{1}$ planes (that is, the $10\bar{1}$ reflection will get weaker), whereas it will tend to enhance the density profile in the $11\bar{2}$ view (that is, the $11\bar{2}$ reflection will get stronger). In fact it was shown very clearly by Huxley (*J. molec. Biol.* 37, 507; 1968) and later by Haselgrove and Huxley (*J. molec. Biol.* 77, 549; 1973) that the intensity ratio ($I_{10\bar{1}}/I_{11\bar{2}}$) of the $10\bar{1}$ and $11\bar{2}$ reflections changed from about 2.5 for muscles at rest, to slightly less than 1 for muscles generating tension and to about 0.25 for muscles put into rigor (all at rest length). The rigor state is known to be one in which a large number of crossbridges are attached to the actin filaments and these observations were taken to mean that fewer crossbridge attachments occur when a muscle is generating tension than in rigor.

Unfortunately, although it is generally true that the more attached crossbridges there are the lower will be the ratio

$I_{10\bar{1}}/I_{11\bar{2}}$, the attachment number is in theory only one of a number of factors which can affect this ratio. Lynn showed (*Biophys. J.* 21, 93; 1978) that the configuration of the attached bridges also has a marked effect on this ratio. Lynn considered two attached states for the myosin heads, one with the long axis of the heads at about 45° to the thin filament axis (as in the rigor state) and one at about 90° to the thin filament axis. His modelling showed that a ratio $I_{10\bar{1}}/I_{11\bar{2}}$ of about, say, 0.5 could be due either to labelling by crossbridges in the 45° configuration of 40% of the actin monomers in the thin filaments or to 20% labelling by crossbridges in the 90° configuration. Since it is now thought that a number of attachment configurations might be involved in each crossbridge cycle in active muscle, it is clear that the intensity ratio of the equatorial reflections will not in general be a good indicator of the number of attached crossbridges. However, provided the distribution of crossbridges among the attachment states is constant, as may well occur in muscles which generate tension at a fixed length (isometric contractions), it is possible then that the equatorial intensity ratio might vary directly according to the number of attached crossbridges and therefore, on present thinking, to the level of isometric tension generated by the muscle. In a recent

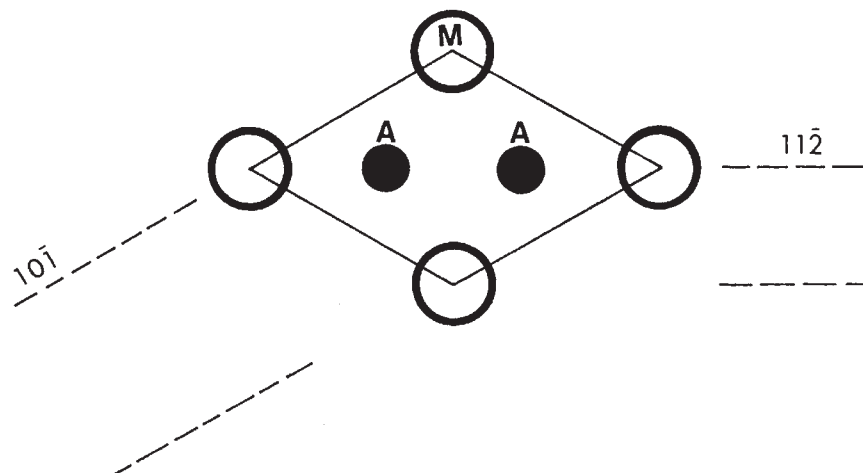


Fig. 1. Unit cell of the hexagonal array of filaments in the overlap region of the sarcomere in vertebrate skeletal muscle. M, myosin filaments; A, actin filaments. The directions of the $10\bar{1}$ and $11\bar{2}$ planes are indicated, in this view of the unit cell down the long axis of the muscle.

paper, Yu, Hartt and Podolsky (*J. molec. Biol.* **132**, 53; 1979) have presented the results of an elegant series of X-ray diffraction experiments in which they have monitored the individual intensities (and the intensity ratio) of the 101 and 112 equatorial reflections from frog sartorius muscles producing different levels of isometric force. They found that the ratio I_{112}/I_{101} (the reciprocal of the ratio discussed above) increased systematically with increasing force (with a slight upward curvature), while the individual intensities either decreased (101 reflection) or increased (112 reflection). The observed variations were said to agree well with theoretical curves calculated assuming two populations of crossbridges, attached and detached, and the attachment number increasing linearly with tension. Although this is clearly an oversimplification, it supports the general contention that more crossbridge attachment should be associated with greater tension generation. This result differs markedly from the previous observations of Podolsky *et al.*, (*Proc. natn. Acad. Sci. U.S.A.* **73**, 813; 1976) on muscles shortening at different speeds under a constant load. In this case it might be expected that both the attachment number and the distribution of attached states would vary with shortening speed, but Podolsky *et al.* found that the equatorial intensity ratio varied very little. It must be concluded either that the current ideas are wrong or that both the distribution and number of the attached bridges change together in such a way that the effect of one is virtually cancelled by that of the other.

One feature of the new results of Yu *et al.* on isometric contractions, not immediately explained by their modelling, was the observation that the intensity of the 101 reflection dropped rapidly from its resting value even at relatively small tensions and then reduced more slowly as tension increased. Yu *et al.* attributed this to some disorder in the filament lattice accompanying the low level of crossbridge attachment, but an alternative addition to the simple two state model is the addition of a third activation step before crossbridge attachment to actin can occur (Squire *A. Rev. Biophys. Bioeng.* **4**, 137; 1975). Such an activation step is suggested by X-ray diffraction results from muscles stretched so that no filament overlap occurs (Huxley *Cold Spring Harbor Symp. quant. Biol.* **37**, 361; 1973; Haselgrove *J. molec. Biol.* **92**, 113; 1975). Here evidence was obtained for changes in thick filament structure on activation of the muscle even though no actin attachment was thought to be possible.

But the detailed interpretation of these results depends critically on the symmetry of the myosin filaments in vertebrate

muscle. The crossbridges are thought to be arranged on a multi-stranded helix around the cylindrical myosin filament shaft. A 3-stranded model is now generally accepted but recent biochemical evidence (Pepe & Drucker *J. molec. Biol.* **130**, 379; 1979) suggests that there may be four strands. However, convincing structural evidence is required to settle the matter finally. Nor is this problem confined to vertebrate muscle. The thick filaments in insect flight muscle, larger in diameter than those in vertebrates, were initially thought to be 2-stranded and then 6-stranded. Now, Wray (*Nature* **277**, 37; **280**, 325; 1979) has suggested, largely on circumstantial evidence, that the number of strands of crossbridges on the insect thick filaments might really be four. It is crucial to our understanding of the mechanism of muscular contraction that the symmetries of these filaments be determined unambiguously. It would then be possible to interpret sensibly the wealth of X-ray diffraction data already obtained from muscles in a variety of states. The known biochemical kinetics of the actomyosin ATPase could then be related more realistically both to the number of attached crossbridges and to the different structural steps involved in the crossbridge cycle. □

Inverse lifetimes and the band structures of solids

from R.H. Williams

AN accurate understanding of the behaviour of electrons in solids and the way that behaviour is related to the chemical composition and crystallographic structure is of considerable importance both fundamentally and technologically. The electrical resistivity of metals and insulators, and the behaviour of many solid state semiconductor devices, for example, depend on the detailed relationships between the energies and the momenta of electrons in those solids and accurate methods of probing the so-called 'band structures' have long been sought. Considerable progress has been achieved by studying such properties as the wavelength dependence of the optical absorption and reflectivity but during the past 10 years the technique of photoelectron spectroscopy has become one of the most promising and widely used. In the most commonly used form of the technique, electrons are emitted from the solid following irradiation by energetic photons and the energy distribution $N(E)$ of those electrons, which is clearly related to the occupied electron states, is measured. Quite recently the power of photoelectron spectroscopy has been vastly increased by the introduction of angle resolving techniques (see, for example, *Contemporary Physics*, **19**, 389; 1978) whereby a movable electron velocity

analyser can probe $N(E)$ for electrons emitted in various directions. Assuming the momentum of the emitted electrons parallel to the surface to be conserved during the emission process then it becomes possible to establish this quantity inside the solid for the various electron states. Indeed for some materials, such as those which have a graphitic or layered structure, such measurements yield a good approximation of the full band structure which for the layered materials is largely two-dimensional in nature (see Larsen, Chiang & Smith *Phys. Rev. B* **15**, 3200; 1977). Interpretation of the experiments, however, may often be made complex due to the presence of the solid surface. The excited photoelectrons are strongly scattered by other electrons with the result that those which emerge without substantial loss of energy originate very close to the surface and may reflect the electronic structure of surface intrinsic or extrinsic (for example, due to adsorbed gases) states. The short inelastic mean free path, or 'escape depth', of photoelectrons leads to a broadening of features in angle resolved photoelectron spectroscopy and as pointed out by Pendry and Titterton (*Communications in Physics* **2**, 31; 1977) it should be possible from the peak widths in such experiments to determine not only the electron and hole lifetimes but also the electron mean free path and its variation with electron energy. This information is of considerable interest in the study of materials by electron spectroscopy.

Recently Eastman, Knapp, and Himpsel (*Phys. Rev. Lett.* **41**, 825; 1978; *Phys. Rev. B* **19**, 4952; 1979) and Thiry *et al.* (*Phys. Rev. Lett.* **43**, 82; 1979) have conducted inverse lifetime measurements of electrons and holes in copper single crystals using angle resolved photoelectron spectroscopy. At the same time the two groups have determined the electronic band structure of copper along well-specified crystallographic directions with unprecedented accuracy. Both groups used the power of angle-resolved photoemission and made use of the great advantages to be obtained by using synchrotron radiation as the light source (the synchrotron at the University of Wisconsin in the case of Eastman *et al.* and that at Orsay, France for Thiry *et al.*). Synchrotron radiation is a near ideal source for such studies as it is intense, highly polarised, and coupled to a suitable optical monochromator produces tunable photon energies over a large wavelength range. Both groups find that their spectra reflect occupied bulk electron states with momentum and energy being conserved in the emission process and by studying emission along a direction perpendicular to the crystal surface they were able to determine the full band structure along that direction. To map out the occupied electronic states it is necessary to have some knowledge of the states into

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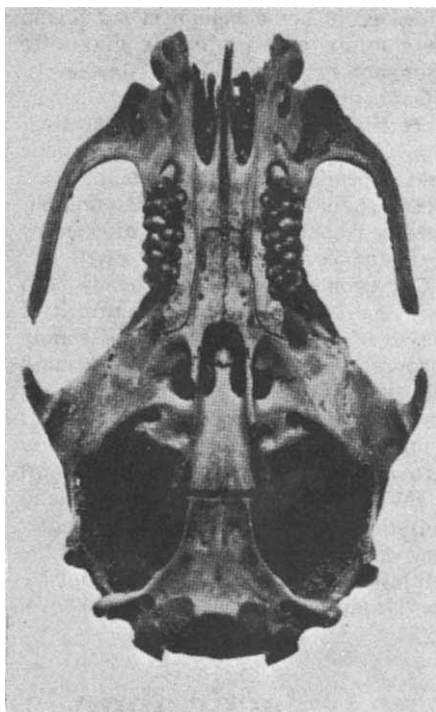
which the electrons are excited and Thiry *et al.* assume that excitation occurs into a free electron final state band. This procedure has previously been shown to give remarkably accurate results for the occupied states of some metals by Stohr *et al.* (*Phys. Rev. B* 17, 587; 1978). Eastman's group makes use of the calculated electron states and both groups obtain very good agreement with theoretical predictions of the band structure of copper. Electron mean free paths determined from the experiments confirm the well known surface sensitivity of photoelectron spectroscopy particularly for excitation wavelengths below about 500 Å. Thiry *et al.* observe excited 3d hole lifetimes in copper which are in good agreement with the estimates of Pendry and Titterton, and further show the great importance of having a high angular resolution in the experiments.

These experiments again underline the power of angle-resolved photoemission coupled to a synchrotron radiation source as an accurate probe of electron states in solids. Studies similar to those described for copper, above, have also been conducted on nickel (Eastman, Himpsel & Knapp *Phys. Rev. Lett.* 40, 1514; 1978) and the ferromagnetic exchange splitting accurately established. Coupled to other variations of the photoemission technique such as photoelectron diffraction (see *News and Views*, 279, 755; 1979), which enable the locations of atoms near a solid surface to be established, an active and rewarding future in this area is certain, particularly as new dedicated synchrotron radiation sources such as the one at Daresbury in the United Kingdom become operational. These new methods will lead to a greater understanding of metals, alloys, semiconductors and insulators and will undoubtedly be important in probing areas such as surface reactions, oxidation of solids, and corrosion. □

The black rat in Britain

THE finding by archaeologists of remains of *Rattus rattus* in a filled-in Roman well in York in the north of England, suggests that the black rat was present in Britain several centuries earlier than had previously been supposed. The finds, which are discussed by James Rackham of the University of Durham in the latest issue of *Antiquity* (53, 112; 1979), lend support to the idea that epidemics of plague occurred in Britain well before the infamous Black Death of the Middle Ages, and may even have been common in Roman times.

Historians have assumed that the black rat — to be distinguished from its close relative the brown or Norway rat, *Rattus norvegicus* — was not introduced into Britain until the Norman period, when returning Crusader ships brought it from the Near East. The many contemporary



Black rat skull found in a Roman well in York.

records of 'plague' in Anglo-Saxon Britain in the 6th and 7th centuries AD have been either ignored or explained away as outbreaks of smallpox or other virulent diseases.

There is no doubt that the high mortalities in the early centuries AD were often caused by a multitude of diseases, of which smallpox was certainly one. But it does not seem likely that Roman Britain would have completely escaped the plague epidemics that swept around the Mediterranean area and across Europe between the 1st and 6th centuries AD. The most serious of these pandemics — without doubt of bubonic plague — began in about AD 540 during Justinian's reign and spread from the Mediterranean up into central Gaul and Germany, and almost certainly would have reached the British Isles.

Plague usually spread from one coastal port to another because infected black rats would find their way on to and off ships. As there was extensive sea trading between Roman Britain and the rest of the Empire the introduction of rats into a British port could easily have happened. York was a thriving Roman administrative and trading centre. Like other large towns of the period, it was a port of call for supplies from many parts of Europe — indeed it is known to have had strong connections with Bordeaux and Rhineland. So it is not surprising that the black rat slipped into the town one day.

The rat remains recovered from the Roman well represent at least two individuals and include a well-preserved skull, a complete mandible with all its teeth, fragments of maxilla and premaxilla, and a cervical vertebra. The wall was timber-lined and was probably built in the

late 2nd or early 3rd century and was then filled-in towards the end of the 4th century AD. During excavation careful checks were made to exclude the possibility that the rats had burrowed into the well after it had been sealed. This, and other evidence from Roman objects found in association with the rat remains and radiocarbon determinations suggest a 4th, or at the latest a 5th century date for the specimens.

These finds of the black rat in Roman Britain lend some support to John Wachter's controversial idea that plague led to the downfall of Romano-British towns in the 5th and 6th centuries AD at the time the Western Roman Empire was disintegrating. Wachter has suggested (*The Towns of Roman Britain*, Batsford 1974; *Roman Britain*, Dent 1978) that the depopulation of these towns was caused by epidemics striking an already threatened Romano-British culture. As economic decline set in, so the way was made easy for the conquering Anglo-Saxons and the start of the Dark Ages.

S.B.

Quest for superfluid hydrogen

from P. V. E. McClintock

THE possibility of preparing monatomic hydrogen (H_1) at high densities seems to have come a stage closer with three papers published in recent issues of *Physical Review Letters*. Two separate research groups have simultaneously reported the first observations of magnetic resonance from H_1 held at liquid helium temperatures: Crampton, Greytak, Kleppner, Phillips, Smith and Weinrib of the Massachusetts Institute of Technology (*Phys. Rev. Lett.* 42, 1039; 1979); and Hardy, Berlinsky and Whitehead of the University of British Columbia, Vancouver (*op. cit.*, 1042). On a slightly different tack, Guyer and Miller of the University of Massachusetts, Amherst, have calculated how H_1 may be expected to interact with a helium coating on the wall of its containing vessel (*op. cit.*, 1754).

The notion of monatomic hydrogen has been around for quite a long time. Hecht seems to have been the first to point out (*Physica* 25, 1159; 1959) that H_1 at low temperatures and high densities might be expected to display quantum properties on a grand scale and, in particular, that it might undergo a superfluid transition rather like that of liquid 4He . These possibilities rest on the observation that, although a pair of hydrogen atoms with oppositely directed electron spins attract each other and form a molecule of H_2 , a pair of atoms with parallel electron spins (in a so-called triplet state) experience a repulsive interaction. This means that a gas of spin-aligned H_1 atoms could be cooled to very low temperatures, even at a high density, without ever condensing into a

liquid, let alone solidifying, as do all other materials except helium (which stays liquid to absolute zero). The weak interaction forces between the atoms, coupled with the very low atomic mass, provide the ideal recipe for quantum behaviour: since the atomic mass is only a quarter that of ^4He , one may guess that the quantum properties of H_1 gas would be even more extreme than those of liquid helium.

Similar ideas can be applied to monatomic deuterium (D_1) and tritium (T_1), the heavier isotopes of hydrogen. Some interesting differences in their properties may be anticipated, however, because H_1 and T_1 , each containing an even number of fundamental particles, are bosons, while D_1 , with an odd number, is a fermion. Thus D_1 might perhaps be expected to behave rather like liquid ^3He , whereas H_1 and T_1 might each be expected to behave more in the manner of liquid ^4He . As gases, however, they would probably all have properties closer to those of ideal Fermi-Dirac and Bose-Einstein gases than the heliums. Such questions are of fundamental interest and importance for quantum mechanics and statistical mechanics, and this is a large part of the reason for the intense interest in seeing whether dense monatomic hydrogen can be made a reality on a macroscopic scale.

Although there has been a good deal of theoretical work on spin aligned hydrogen, with detailed predictions for many of its properties, relevant experiments have been somewhat thinner on the ground. The fundamental practical problem is, of course, to find a way of stabilising the H_1 gas against recombination to form H_2 molecules. The basic idea is to use a suitable container placed in an intense uniform magnetic field and held at a very low temperature. The field will induce a splitting in the ground state energy levels of the H_1 atom, depending on whether its electronic spin is pointing with or against the field; and it is hoped that the low temperature will ensure that the atom stays in the lowest level, that is, spin-aligned. It is believed that a field of 10 T and a temperature of about 0.1 K might provide the necessary conditions for stable confinement of the H_1 gas. These conditions are realisable using existing cryogenic technology.

Before a full scale apparatus can be designed, however, it is necessary to obtain information about the properties of individual H_1 atoms at low temperatures and, in particular, about any mechanisms able to cause the spin flips which would rapidly lead to recombination to ordinary H_2 . That is why the work reported from MIT and UBC is so important. Both groups have succeeded in conducting H_1 atoms down to experimental chambers

immersed in liquid helium at 4.2 K and have managed to observe magnetic resonance between the hyperfine levels. This has now opened the way for further, more detailed, studies aimed at measuring, for example, the temperature and magnetic field dependences of the recombination rate. It should also be possible to find out how the recombination rate is affected by the material of the walls of the vessel.

One problem which had already been foreseen is that magnetic impurities inevitably present in the walls may encourage spin flips. A possible way round this difficulty would be to coat the walls with a layer of superfluid ^4He , which is completely non-magnetic. The question then arises as to whether the H_1 atoms might be able to enter the ^4He layer, and thus still be able to reach the walls; whether they will be trapped on the surface of the ^4He layer; or whether they will stay outside the ^4He layer altogether. Miller has shown

(*Phys. Rev. B18*, 4730; 1978) that the H_1 is actually totally insoluble in liquid ^4He ; Guyer and Miller now demonstrate that there will, in fact, be a shallow physisorbed state for H_1 just outside the ^4He layer, but that this state will not be heavily populated. They comment that ^3He impurities in the ^4He could give rise to difficulties since these, too, will congregate at the surface. This potential problem is easily circumvented however, because techniques have recently become available for the virtually complete removal of the 2×10^{-7} parts of ^3He found in commercial ^4He . So it looks very much as though a coating of isotopically pure superfluid ^4He will constitute an effective method for preventing wall-induced spin-flip processes.

Clearly, there is still a long way to go before superfluid hydrogen becomes a reality, but the present flourish of activity aimed in this general direction is most encouraging. □

The Galactic abundance gradient

from M.G. Edmunds

A CONSISTENT picture is at last beginning to emerge of the behaviour of the overall abundance of the elements, relative to hydrogen, as a function of position in our Galaxy. To a good approximation we can regard most elements heavier than helium as being produced in constant relative amounts during a single process — supernova nucleosynthesis — and the abundance of any one element is a fair indicator of the abundances of all the elements. There are of course a few exceptions, elements which are produced during quiescent stellar evolution such as nitrogen, but the majority are believed to come from supernovae. We can regard the Galaxy as a two-component system, made up of a disk of stars and gas, surrounded by a more-or-less spherical halo of old stars and globular star clusters. The pattern of the variation in abundance with distance from the Galactic centre in both disk and halo has become clearer with the publication of several papers over the past year.

K.A. James (*Astrophys. J. Suppl.* **39**, 135; 1979) has used narrow and wide band photometry to estimate metal abundance (anything heavier than helium) in a sample of giant stars and about 40 star clusters in the disk. Photometry can yield an abundance for a star because the strength of the absorption lines in the spectrum of the star's atmosphere is dependent on abundance, and the lines are not evenly distributed in wavelength. Thus a star with high abundances will emit less light in certain regions of its spectrum (particularly at short optical wavelengths), and more light in other regions, than will a metal-poor star. The differences are large enough to be detected by photometry of selected

wavelength bands. The study of clusters is particularly useful because their distances can be estimated with reasonable accuracy, something that is very difficult to do for individual stars.

James finds there there is a distinct gradient of abundance decreasing outwards from the region of the Sun (10 kpc from the centre) to as far as he has measured at 14 kpc, although inwards towards the Galactic centre (from 10 kpc to 8 kpc) he claims to find little or no gradient. But his results are open to reinterpretation, and it may simply be that the classical notation for presenting abundances (as the logarithm of the quotient of the metal abundance relative to hydrogen in a star divided by the same thing for the Sun) may have obscured the actual trend. If James's data are replotted on a linear abundance scale, rather than a logarithmic one, then a simple straight line fits beautifully within the error bar in the plot of abundance against distance from the centre, both inside and outside the position of the Sun.

This therefore implies a simple linear abundance decrease in going from 8 to 14 kpc, a result which is in nice agreement with the indications of a linear abundance gradient in the interstellar material, deduced by observation of emission spectra of HII regions. There is much scatter in these derived nebular abundances, although this is probably mainly due to errors in observation and analysis, and it had originally been thought that this latter gradient was much steeper. But a reanalysis of the spectra assuming that temperature fluctuations in the nebulae are not important (as seems reasonable from recent theoretical modelling) gives a gradient in oxygen

abundance in close agreement with this reinterpretation of James's result. (The reanalysis of the HII regions is discussed by Pagel in *Stars and Stellar Systems* ed. Westerlund, Reidel, 1979.)

If these gradients stay linear to the centre of the Galaxy, they imply a central enhancement over the solar neighbourhood by a factor of $\times 2.1$ as judged by the 'metals' gradient, and a (negligibly different) factor $\times 2.5$ as judged by the oxygen gradient. It may be dangerous to extrapolate so far, but interstellar obscuration makes optical observations in the disk difficult beyond 8 kpc. The extrapolation is anyway in encouraging agreement (within the probable errors) with a determination of heavy element abundances of about three times solar neighbourhood deduced by infrared observations of nebular lines from the Galactic centre (Aitken, Griffiths and Jones, *Mon. Not. R. astr. Soc.* **176**, 73p; 1976). Thus a linear abundance gradient in both stars and gas is indicated, with a factor of 2-3 decrease between the centre and the Sun. This conclusion will be of considerable value in improving estimates of the total gas content of the disk which rely on indirect derivations of H_2 abundance from millimetre-wavelength observations of co-existing carbon monoxide molecules. The interstellar CO abundance should depend linearly on the carbon abundance, given that the Galactic O/C ratio exceeds unity, and determinations have usually assumed a constant C/H ratio (fixed by observations near the Sun) throughout the disk. The H_2 content of the disk may thus be somewhat lower than previously thought.

For the halo, the situation is more uncertain. One of the principal components is the globular cluster system, and controversy has arisen over the existence or non-existence of a correlation between the overall metal abundance of a cluster and its distance from the Galactic centre. Undoubtedly there exists a great range in abundances for clusters at any given distance, but while Searle and Zinn (*Astrophys. J.* **225**, 357; 1978) claim — mainly on the basis of photometry — that there is no overall gradient, Harris and Catena (*Astrophys. J. Lett.* **231**, L19; 1979) — partly on the basis of a compilation of high-dispersion spectral analyses — claim that there is. Comparison of abundances when stars have been analysed in different ways is always difficult, and resolution of the controversy may have to wait until a complete homogeneous set of analyses is available. The common ground between the two investigations is (1) there is a range in metal abundance of the clusters from solar to 1/100 of solar at all radii out to 10 kpc, and (2) there are no 'metal rich' clusters between 10 and 30 kpc, but a range between 1/10 and 1/100 of solar at any

radius in this region. Beyond 30 kpc Searle and Zinn's sample is very small, but Harris and Catena claim abundances less than or equal to only 1/100 solar out to 100 kpc. They include in their sample the dwarf outlying Draco and Ursa Major systems which are rather more open structures than globular clusters but almost undoubtedly part of the Galactic system. Searle and Zinn argue that, apart from the metal rich clusters which may have formed late in the early evolution of the Galaxy, a lack of gradient is apparent. This, together with the particular form of the spread of abundances at all radii, would indicate that the clusters essentially formed independently as stellar systems, lost gas, and then came together to form the halo. If Harris and Catena's interpretation is preferred, in that the mean metal abundance at 50-100 kpc is lower than at 10-30 kpc and hence implying some gradient, then the assumption of independence would have to be modified so that the halo had some overall structure before and during formation of the clusters — rather than just aggregating together afterwards.

In the currently popular model, the Galaxy is presumed to have collapsed from a more dispersed state to its present form under the influence of self gravity — a model which, as Searle and Zinn point out, was first proposed by Kant as long ago as 1755. Abundance gradients can be produced easily in reasonably sophisticated computer simulations of the evolution of this model, but exactly what is the dominant process which causes the actual Galactic gradient is still not clear. Analytically, a linear gradient can be understood with the simplest possible model for synthesis resulting from converting gas into successive generations of stars, provided particular (but not unreasonable) radial distributions of gas and star mass are assumed (Pagel, *op. cit.*). But such a model does not give the observed unexpectedly small number of old stars in the disk with low abundances, and radial gas flows during the evolution of the Galaxy will probably have to be involved. The wide spread of abundances in the halo clusters certainly points to a fairly chaotic evolution there. Better knowledge of the spatial variation of element abundance in our own and nearby galaxies is certain to place useful constraints on our ideas of the formation and evolution of galaxies. □

Erratum

A line was accidentally omitted from the contribution of W.L. Fink and E.O. Wiley in a recent *News and Views* (*Nature* **280**, 542; 1979). In the fifth paragraph, the sentence beginning on the eighth line should read: "The lineage is broken up, naturally and non-arbitrarily, by the many speciation events which have occurred from the Devonian to the Recent."

Moas as rockhounds

from Ian Smalley

THOUSANDS of years before the first human set foot in New Zealand, the now extinct, flightless bird the moa, was roaming the countryside searching for semi-precious gemstones such as agate, opal, chalcedony, chert and jasper. It swallowed suitably sized pebbles of these materials and retained them in its gizzard to grind up its food. The birds carefully chose the hardest available stones and when they had worn smooth they spat them out — for latter-day geologists to find. B.W. Hayward of the New Zealand Geological Survey has reached this conclusion after studying the pebbles found on the sandy hills surrounding the Auckland University Field Club scientific station at Kawerua, on the west coast of Northland (*Tane* **20**, 159; 1978). The pebbles can only have come from the Waimamaku Valley some 5-10 km away; those examined had a mean maximum diameter of 28 mm and were highly polished and rather flattened in shape.

The birds seem to have been highly selective and to have concentrated on collecting and swallowing only the hardest of the local stones; these are the ones which, largely because of their hardness, fall into the semi-precious category. Before the arrival of man there must have been vast numbers of moas roaming New Zealand and they have left widespread and abundant deposits of used gizzard stones. In fact it seems that moas were common enough to have significantly affected the nature of the New Zealand vegetation, and the plant types seen today show the effects of long-term moa browsing (Greenwood & Atkinson *Proc. N.Z. Ecol. Soc.* **24**, 21; 1977). The gizzard stones are virtually always varieties of quartz and exceptions have been observed in only two localities. The moas found in the well-known deposit at Pyramid Valley, North Canterbury used greywacke stones. This valley passes through Tertiary limestones and soft sandstones with greywacke exposed in the headwaters. There seems to be no nearby source of quartz and the next most resistant rock type available is greywacke; a similar situation exists for the stones from near Lake Tekapo.

At Kawerua one of the richest localities for finding pebbles is around the actively eroding Pukenuiorongo Bluffs. The Bluffs are 120 m above sea level and consist of a series of large washouts exposing a 40 m thick sequence of Pleistocene sands. This sequence probably accumulated on land as there is no evidence of water deposition

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and although none of the sandstones preserves any laminations or crossbedding typical of dune deposits, they are probably wind blown accumulations having advanced inland from the coast. The stones were presumably coughed up by the moas in the sandy coastal areas and buried by the advance of the dune sand.

The pebbles collected from Pukenuirongo Bluffs are generally flatter and more rounded and polished than most of the authenticated moa gizzard stones which are usually found in association with moa skeletons. This supports Hayward's hypothesis that the collected pebbles were supplied by live moas who coughed them up when their grinding efficiency was significantly reduced. Hayward also proposed that the moas sought actual rock types — even though the majority of the quartzose pebbles are gleaming white and easily distinguished the moas detected and swallowed numbers of brown, red and black pebbles of similar lithologies. It seems that the birds made their selection after a careful mineralogical assessment. □

Evidence for gluons?

from Frank Close

THE latest results from PETRA, the Hamburg electron positron colliding ring, appear to add considerable weight to the quantum chromodynamic theory of the interquark force. Quarks are hypothesised to possess a new form of charge, known as colour, and interact with one another by exchanging coloured gluons (this process being somewhat analogous to the way that electrically charged objects mutually interact by exchanging photons). Just as the photon is the quantum of the electromagnetic force so are coloured gluons the quanta of the chromodynamic or interquark force. The theories of these forces are respectively quantum electrodynamics (QED) and quantum chromodynamics (QCD), the similarity in their names bearing witness to the similarity in their mathematical structures.

When electrons are annihilated by their antimatter (positrons) the energy is converted into light (this is well described by QED). This light lives for only a fraction of a second before being transformed into particles. Sometimes the light reconverts into leptons and antileptons (like the electron and positron). Alternatively the light may become a quark and an antiquark (q and $\bar{q}$). This process is conventionally denoted

$$e^+e^- \rightarrow \gamma \rightarrow q\bar{q}$$

So far everything is described by QED. In both cases electrical charges are being accelerated (positive and negative have been produced out of nothing) and QED predicts that quanta (photons) will be radiated by the produced charged particles



(the lepton pair or the quark and antiquark). This is indeed the case experimentally and has been well understood for many years.

QCD for colour carriers is analogous to QED for charge carriers. Therefore it may be no surprise to learn that when colour charges are accelerated the theory predicts that gluons will be radiated. Quarks and antiquarks carry colour and so the real process is

$$e^+e^- \rightarrow \gamma \rightarrow (q + \text{photons} + \text{gluons}) + (\bar{q} + \text{photons} + \text{gluons})$$

From established QED we can calculate how much of the signal is due to the photon radiation. This then makes possible a study of the characteristics associated with gluon radiation from coloured quarks and comparison with the QCD predictions. There are three qualitative types of phenomena expected, one of which was only becoming apparent earlier this year (*Nature* 277, 349; 280, 450) and the third of which now appears to be showing up in the most recent Hamburg experiments.

(1) It is possible that the quark and antiquark may bind together and form a vector meson, like the J/ψ or Υ mesons, until such time as they annihilate one another thereby causing the meson to die. QCD predicts two things here. First, the probability for the quark and antiquark to annihilate decreases as the mass or energy of the $q\bar{q}$ bound state increases. This is apparently seen in that the J/ψ (3.1 GeV) and Υ (9.5 GeV) have anomalously enhanced lifetimes compared to what would have been expected in the absence of QCD based on the known lifetime of the lighter ϕ (1.0 GeV).

Second, it makes predictions as to the distribution in energy and space of the particles that are produced in the decay. The theory requires that the quark and antiquark annihilate, thereby producing three gluons. These gluons then transmute into pions and kaons that are detected by the experimental apparatus; three jets of particles should be detected bearing witness to the memory of the three gluons.

The J/ψ at 3 GeV is too light to allow a good test of this prediction. Studies of the more massive Υ early in 1979 began to provide data supporting the three-jet prediction (*News and Views* 277, 349; 280, 450). The best tests of this prediction may have to wait until (if?) a yet heavier vector meson (θ) is discovered, a bound state of the hypothesised sixth quark (t and $\bar{t}$). Such



a meson, (if it exists) appears to be too massive to be produced at the present generation of electron-positron machines.

(2) The energy of the electron-positron annihilation might not be suitable for a vector meson to be simply produced. In this case the $q\bar{q}$ and associated glue are radiated off away from one another.

QCD predicts that, at high energies, the most likely configurations are where the quark and antiquark carry off most of the energy and the glue is either very soft or highly collimated along the $q\bar{q}$ axis. As a consequence two jets of particles will be seen in the detectors.

Such 'two-jet events' have been known to exist for about 5 years. QCD predicts that as the electron-positron annihilation energies increase, so these two-jet events will become increasingly collimated and pencil-like. Data at the highest energies indeed support this.

(3) Although these jetlike — pencil — events should dominate, there is a chance that the quark (or antiquark) radiates a hard gluon which carries off momentum and deflects the quark (antiquark) from its path (Fig 1a). If resolution is poor this will show up as a thin-jet plus fat-jet event, like a squash or tennis racket (Fig. 1a). Evidence for such events was reported by the TASSO collaboration at the CERN conference in June.

The latest results from PETRA are obtained with it operating at the highest energy of 32 GeV. The events are dominantly two-jet in character but a significant number of planar tennis racket type were seen. Some of these do appear to be resolvable into a genuine three-jet structure (Fig 1c) (known as propeller or Mercedes events — the German laboratory inspired by the manufacturers symbol). These latest results were announced at a conference at Fermilab, Chicago during the final week of August.

So it appears that, at least qualitatively, the behaviour of high energy electron-positron annihilation does mimic the expectations of QCD, the theory of the interquark force. Theorists and experimentalists suspect that these three-jet events are due to a quark radiating a gluon before the detected particles are created Figure 1b shows the $q\bar{q}$ gluon state, which then becomes particles like pions and kaons by an unknown mechanism. However, there is much quantitative work still to be done by experimentatists, and theorists will be trying to find more precise tests. With more work at the highest energies, PETRA seems well set to be able to perform a significant test of the theory. □

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review article

Structure and evolution of the Moon

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The scientific investigation of the samples returned by the Apollo and Luna missions, together with geophysical and photogeological data, has provided a reasonably complete understanding of the structure, composition and evolution of the Moon, and has placed many constraints on theories of its origin.

THE tenth anniversary of the first of the manned Apollo missions (20 July 1969) to the Moon provides an appropriate opportunity to review the current state of our knowledge of the structure, composition, evolution and origin of the Moon. Much of our scientific understanding stems from the samples obtained during the Apollo missions. Coupled with the various geophysical and photogeological information also obtained, and reinforced by the unmanned missions to Mars, Mercury and Venus, the result has been the development of the science of planetology. Insights into the origin of the Solar System, the composition of the solar nebula, and the relation of the Earth to the other members of the inner parts of the Solar System have all been obtained. The current state of lunar and planetary science is illustrated by over 50,000 pages of literature written since 1969. The latest additions to this mountain of data are the 1,426 pages of abstracts of papers for the tenth Lunar Science Conference held in Houston, Texas, in March 1979. Within the limits of this survey, it is not possible to reference adequately all this material, or to do justice to many of the important scientific results. Some critical and interesting areas (soils, track studies, rare gases, and meteorite flux) have had to be excluded because of space limitations.

Internal structure

Models for the internal structure of the Moon down to depths of 1,000 km have now reached a consensus (Fig. 1). The lunar crust, which is less dense than, and chemically distinct from the interior, varies in thickness from 60 km to 100 km. These values are constrained partly by density¹ (crustal density = 2.96 g cm^{-3}), but also by some seismic evidence for the base of the crust^{2,3}. The mare basalts, although prominent visually, constitute less than 1% of the crustal volume (0.1% of lunar volume). The highland crustal thickness is 60 km at the Apollo 12 site and 75 km at the Apollo 16 site. A 20-km discontinuity within the crust does not reflect the base of the mare basalts, but rather the depth of fracturing or of closure of cracks produced by the large collisions, thus representing the change from fractured to competent rock^{2,3}.

The upper mantle or lithosphere extends from the base of the crust (60–100 km) to 400–500 km. The average P wave velocity (V_p) is 7.7 km s^{-1} and the average S wave velocity (V_s) 4.45 km s^{-1} , with negative velocity gradients. There is some inconclusive evidence for lateral heterogeneity⁴. A minor discontinuity exists^{2,3}, most probably located at a depth of 480 km, with a change to lower seismic wave velocities (V_p 7.6 km s^{-1} ; V_s 4.2 km s^{-1}) down to depths of about 1,000 km. Deep within this

zone (800–1,000 km depth) occur most of the moonquakes. A few (so called high frequency teleseismic events) occur at depths of about 100 km, most probably associated with deep fractures surrounding the ringed basins⁵. The crust itself is aseismic. Below 1,110 km, the structure becomes less certain. P and S waves become attenuated (V_p $\sim 5.0 \text{ km s}^{-1}$; V_s 2.5 km s^{-1}), consistent with the presence of a small amount ($\sim 1\%$) of melt. There is a major discontinuity at about 1,110 km, with a very sharp decrease in S wave velocities^{2,3}.

A lunar core? The possible existence of a lunar core has been much debated but the evidence for it remains inconclusive. The seismic data restrict the core radius to 170–360 km (less than 2% of lunar volume)⁶. The lunar coefficient of moment of inertia⁷ constraint ($1/MR^2 = 0.391 \pm 0.002$) requires a density increase in the deep interior, in addition to the low density crust. Although such a constraint may be satisfied by a core, it is also satisfied by a density increase at the 480 km discontinuity to a more iron-rich interior^{2,3}.

The restrictions on core size to about 2% of the lunar volume make such a core of little geochemical significance, for example as a sink for the siderophile elements. The presence of a core implies complete lunar differentiation. Most of the evidence (discussed in later sections) favours initial melting of half or two-thirds of the Moon rather than a completely molten body.

Whether such a small core could have generated a magnetic field, via conventional dynamo action, remains uncertain, as do the origin and intensity of the ancient lunar magnetic field. The surprising initial observation that the returned lunar samples possessed a stable natural remanent magnetisation (NMR), which indicated the former presence of a now vanished magnetic field, has continued to baffle investigators. Considerable efforts have been made to determine the palaeointensity of this field, and there is general agreement that fields up to 1 Oe were required on the lunar surface to account for the NMR^{8–10}. Some evidence exists for a decay in field strength over about two orders of magnitude between 4,000 and 3,000 Myr but the evidence, particularly from the breccias, is still a matter of controversy^{9,10}. This is leading to a concentration of effort on the mare basalts, which possess a much simpler history than the complex breccias¹⁰. Debate continues about the local or Moon-wide nature of the field¹¹. Resolution of this problem may have to await a future lunar orbiter. The locally varying magnetic anomalies observed by the orbiting subsatellites during the Apollo 16 mission definitely correlate with ejecta blankets from the ringed basins and older large craters¹². The youngest identifiable area yielding such an anomaly is the feature Reiner gamma, dated stratigraphically at 2,900 Myr.

The origins proposed for the lunar magnetic field encompass a large number of processes, which fall within the following categories: (1) the field is random due to localised near-surface origin¹³; (2) external uniform fields; (3) internal dipole field⁴. No resolution seems possible at present between these alternatives¹⁰. Note that attempts to relate the lunar field to the presumed interplanetary field responsible for the fossil magnetism observed in meteorites may be misleading. Among other caveats, the fields responsible for the magnetic effects in meteorites were in existence about 1,000 Myr earlier than those responsible for the lunar magnetism¹⁴.

The lunar highlands

Crustal densities, based on orbital γ -ray data for iron, are consistent with models of uniform density and variable crustal thickness (Airy-type model) rather than the reverse (Pratt model¹⁵). Contrary to earlier interpretations¹⁶, the Apennine Mountains, rising 7 km about Mare Imbrium, may be isostatically uncompensated, supported by the lunar crust and lithosphere for 3,900 Myr¹⁷. Coupled with the mascon data, this provides a constraint on lunar thermal models and on the strength of the lunar interior. Partial melting in the interior, which produced the mare basalts as late as 3,200 (or 2,600) Myr, did not weaken the upper lithosphere sufficiently to allow isostatic compensation. Younger large lunar craters (for example, Copernicus) show negative gravity anomalies (that is, net mass deficiency) but older craters such as Humboldt do not¹⁸. The possibility that the older craters are isostatically compensated is not consistent with the presence of the uncompensated Apennines. Possible explanations include the reduction of porosity in the subcrater structure by shaking during the Imbrium and Orientale basin forming collisions at 3,900 Myr, or the intrusion of mare basalt as sills and dykes into the fractured subsurface structure.

The large ringed basins (with up to five concentric mountain rings as for Orientale) owe their origin to collisions of asteroids or planetesimals. The energy release during the Imbrium collision has been estimated¹⁹ at about 3×10^{33} erg (corresponding to the impact of a 100-km diameter asteroid of density 3 g cm^{-3} travelling at 16 km s^{-1}), about two orders of magnitude less than the energy required to disrupt the Moon¹⁹. Opinion has been divided between an origin of the outer rings by the initial cratering episode or by later slumping along concen-

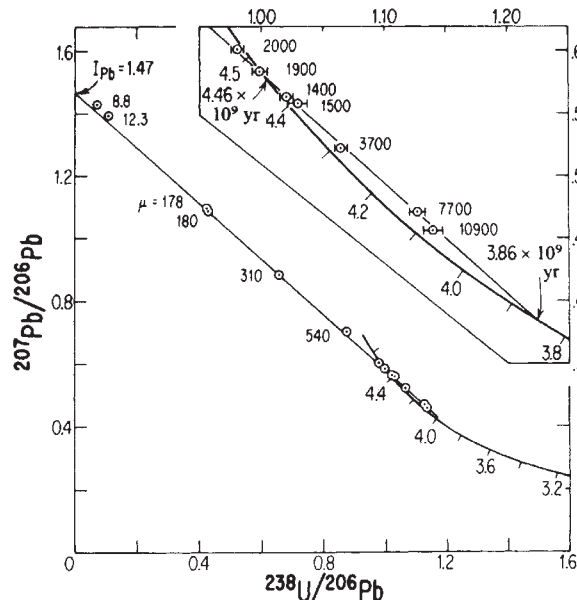


Fig. 2 U-Pb evolution diagram for highland breccias²⁸. Inset shows data points near concordia curve with error bars. The measured value of $\mu = {}^{238}\text{U}/{}^{204}\text{Pb}$ is given for each sample. Of 13 data points, 10 lie precisely on a line intersecting concordia curve at 4,460 Myr and 3,860 Myr. The upper intersection represents the time of early lunar differentiation and crust formation. The lower intersection corresponds to a time of intense bombardment of the Moon (terminal lunar cataclysm). Samples whose data points do not plot precisely on the line may have impact ages slightly different from 3,860 Myr. The cataclysm affected almost all lunar highland rocks, causing severe U/Pb fractionation in the breccias. This is reflected by the linear distribution of the data points on the graph and the strong correlation of μ values with position of the data points. (Courtesy F. Oberli and G. J. Wasserburg.)

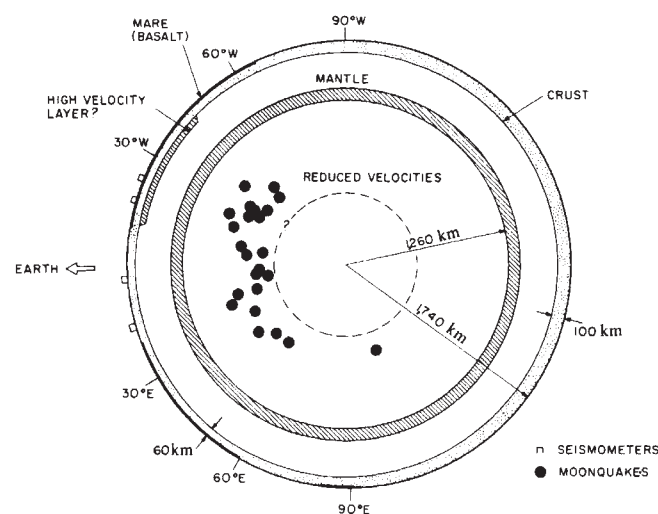


Fig. 1 Internal structure of the Moon^{2,3}, consisting of a 60–100-km thick crust overlying a two-layered mantle, or lithosphere, with a transition zone between 400 and 480 km. Epicentres of typically observed moonquakes are shown. The inner region, within the dashed circle, shows a sharp decrease in P and S wave velocities. (Courtesy N. E. Goins.)

tric faults. The present view based on cratering experiments²⁰ is that the rings formed, like nested craters, during the impact^{21–23}.

The depth to which the impacts which produced the giant ringed basins have excavated and overturned (and thus sampled) the crust remains uncertain. Estimates have ranged from minimum values²⁴ of 8–27 km to depths exceeding the thickness of the lunar crust²⁵. An upper limit is provided by the lack of material collected from beneath the feldspathic crust. The dunite (72415) which is sometimes so described has a mineralogy indicative of shallow, rather than deep, origin²⁶. It may have originated either as a cumulate, crystallising in a pool of impact melt, or, more probably, on account of its old age (4,600 Myr), from liquid trapped in the feldspathic crust, or possibly in a frozen primordial crust. It is thus of doubtful use as an index of the composition of the lunar mantle.

The mechanics of formation of the giant ringed basins are critical for the interpretation of the samples returned from the lunar highlands. Sampling of the 'light plains' units in the highlands (at Apollo 16) demolished the theory that they were of volcanic origin. They are due to the widespread transport of debris across the lunar surface, during the excavation of the giant ringed basins. However, the primary basin ejecta does not blanket the pre-existing surface for more than a short distance from the basin rim. The secondary projectiles flying out from the explosion plough up the surface and create their own local ejecta. The result is that the light plains units are mainly composed of locally derived debris. Thus, the ejecta blanket from the Imbrium collision, which was sampled at the Fra Mauro site during the Apollo 14 mission, although intensely brecciated, does not show the effects of the more than 200 kbar shock pressure which existed at the centre of the Imbrium basin during impact²⁷. The orbital γ -ray and X-ray fluorescence data show much variation across the highlands, consistent with local chemical variability and with the above model.

The date of the formation of the lunar highland crust has been refined as a result of lead isotope investigations (Fig. 2). These isotopic data show two ages, one at 4,460 Myr, which is interpreted as the age of formation, and a second at 3,860 Myr consistent with the age of the Imbrium collision²⁸. These new dates place the crystallisation of the highland crust a little earlier than the 4,400 Myr previously accepted. As the date for the condensation of the Solar System is usually given as 4,570 Myr²⁹, accretion of the Moon followed by melting and crystallisation of at least half the lunar volume, was accomplished very quickly.

The overall composition of the lunar highland surface is given in Table 1. It is highly fractionated with respect to either primitive solar nebula compositions or to the bulk composition of the Moon (Table 1). Although early workers were so impressed by this refractory alumina-rich composition as to suggest a late, heterogeneous accretion episode to account for it, all geochemical and petrological evidence now links the highland crust to the rest of the Moon. For example, the similarity in the ratios of volatile/refractory elements (for example, K/Ba, K/Zr, K/U) in all returned lunar samples³⁰ indissolubly links the highlands and the mare samples derived from the lunar interior.

The question of whether a feldspathic crust would float over a crystallising 'magma ocean' was resolved by experimental results³¹ which demonstrated that such flotation would occur in the anhydrous lunar environment in liquids parental to the highland crust. No report of water on the Moon, even at the level of parts per 10⁹ (p.p.b.), has been substantiated. This contrasts with the terrestrial situation, where the presence of small quantities of water causes the feldspar to sink. This, among other factors, invalidates the case for an early anorthositic crust on the Earth^{32,33}.

Detailed models for the crystallisation of the magma ocean³⁴⁻³⁶ indicate that such processes were complex, probably involving the formation of 'rockbergs' (analogous to icebergs), cycles of assimilation and mixing, and trapping of interstitial liquids to produce the complex suite of crustal rocks. It is probable that an extensive bombardment continued throughout the formation of the crust and for the next 500-600 Myr, culminating in the terminal cataclysm at about 3,900 Myr to which the Imbrium and Orientale basins bear witness. The complexity of the resulting rock types has led to a literature too extensive to review here (see, for example, ref. 37).

In addition to the feldspathic (anorthositic) and primitive Mg-rich components in the lunar crust, a third, known as KREEP, is pervasive. Its origin has been widely debated since its discovery at the Fra Mauro site during the Apollo 14 mission (following traces reported from the Apollo 12 landing site). The continuing bombardment which pulverised the crust has destroyed most of the evidence. One view considers that the KREEP basalts are derived from flows representing pre-mare volcanism²⁷. The absence of identifiable rocks (except for those crystallising from impact melts) makes this view, based on photogeological interpretation, less likely than the other extreme, which relates the origin of KREEP to the crystallisation of the magma ocean. In this model, the extremely high concentrations of such components as K, rare earth elements (REE), P, Ba, Th, U and Zr are derived from the residual melt following crystallisation of the magma ocean³⁸. This model is reinforced by the Sm-Nd systematics³⁹ which indicate a uniform origin for KREEP at the different landing sites, and by the parallel REE patterns independent of total REE abundance which typify many highlands samples⁴⁰.

The mare basalts

Such models for the origin of the crust are related to the question of the source of the dark mare basalts, which have been remarked on in most mythologies. They cover 17% of the lunar surface, although only rarely (in the centres of ringed basins) exceeding thicknesses of 1 km (refs 41, 42). Their concentration on the Earth-facing hemisphere, where the crust is thinner (Fig. 1) is probably a consequence of non-uniform crustal thickness⁴³. The mare basalts are derived by partial melting from depths of 100-400 km. Hence, they provide, in principle, information about the composition of the deep interior and so the nature of their source regions has been keenly debated.

Several recent reviews have appeared⁴⁴⁻⁴⁷ which delineate nearly 20 types ranging from high titanium to very low titanium (VLT) basalts. Most of these types cannot be related simply by partial melting or by fractional crystallisation from a single parent magma, but demand differing source compositions. The nearly ubiquitous depletion in Eu demonstrates prior removal from the source regions of plagioclase. Isotopic systematics (Rb-Sr and Sm-Nd) (see, for example, ref. 48) indicate crystallisation of the source regions at or before 4,400 Myr, hundreds of millions of years before their eruption. Dated flows extend

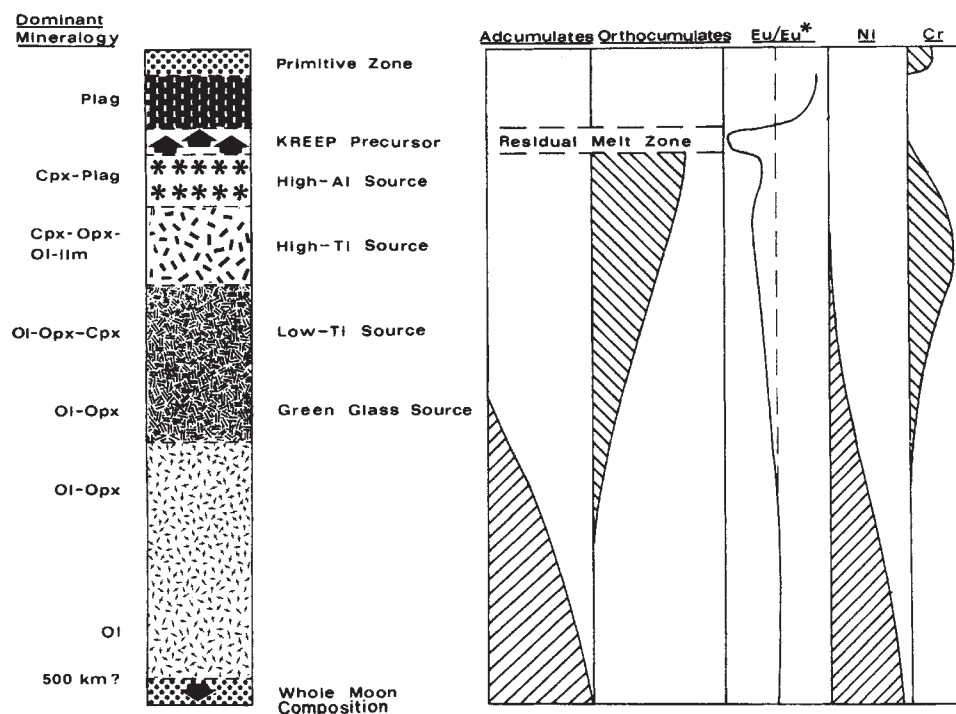


Fig. 3 Schematic diagram to illustrate formation of a mineralogically zoned source region for the lunar mare basalts, during crystallisation of a 500-km deep magma ocean. Adcumulus growth (that is, little trapped pore liquid) predominates at depth, with orthocumulus growth (incorporation of trapped interstitial liquids) occurring at higher levels. The Eu anomaly (Eu/Eu^*) increases to a maximum negative value in the KREEP precursor zone, and is positive in the floating plagioclase crust. Nickel is concentrated in olivine-rich zones in the deep interior. Chromium, possibly mainly divalent^{61,62}, is concentrated in the source regions of the mare basalts. (Adapted from ref. 57.)

from 3,900 Myr to 3,160 Myr⁴⁰. Mare volcanism probably began earlier, before the final cataclysmic collisions, both from photogeological studies⁴⁸ and the presence of basalt clasts in Apollo 14 breccias⁴⁰. The style of eruption is analogous to terrestrial plateau or flood basalts. The combination of low viscosity and high flow rates⁵⁰ produced the characteristic smooth (± 150 m) surfaces of the mare basalts⁵¹. The impacts which formed the large basins, although producing some local melt⁵², did not initiate the floods of basalt from the lunar interior. These were erupted up to hundreds of millions of years after the great collisions. Among the new types of basalts^{47,53-55} described recently, the most significant are the Apollo 17 VLT basalts⁵⁵. These display the high V and Cr and low Ni contents typical of most mare basalts, but in addition have heavy REE (HREE)-enriched patterns, rather than the flat or depleted patterns common to other species. The REE patterns, which include a negative Eu anomaly, preclude derivation either from a primitive interior, or by assimilation of crustal or cumulate material⁵⁵.

When contemplating the whole spectrum of basalt types, derivation from a cumulate source region remains the most promising model. The crystallisation of the magma ocean produces a layered series of differing mineralogy, ranging from olivine and orthopyroxene at the base to clinopyroxene-ilmenite cumulates at shallower levels (Fig. 3). Trapping of interstitial liquids, convective overturn, magma mixing and other processes³⁵ will greatly complicate the simplified models originally envisaged³⁸ but the overall concept accounts for both the isotopic systematics and trace element abundances. The total absence of water and other volatile components means that small-scale heterogeneities in the lunar interior will remain frozen in. A consequence is that mare basalt compositions (even the most primitive such as Apollo 15 green glass⁵⁶ or Apollo 17 VLT basalts⁵⁵) are derived from fractionated source regions and do not provide direct information on the primordial lunar composition.

Much progress has been made in mapping mare basalts from the orbital chemical data⁵⁸ and by remote sensing techniques⁵⁹. The youngest mare basalts, identified in Western Oceanus Procellarum by photogeological techniques, are about 2,600 Myr old⁶⁰. After this time, no lavas reached the surface. The melting of mare basalts in the interior was fuelled by trapped K, U and Th, although most of the lunar budget for these radioactive elements was concentrated in the crust by the early differentiation.

A frozen surface. The cessation of mare basalt flooding at 2,600 Myr caused the last discernible changes to the lunar surface (Fig. 4) except for the production of younger impact craters, such as Copernicus, Kepler, Aristarchus and Tycho. The last major structural change to the lunar surface occurred at 3,900 Myr with the production of the 900-km diameter Orientale ringed basin, with an energy release¹⁹ estimated at 1.4×10^{33} erg. Since that event, the lunar surface shows no evidence of major tectonic activity, expansion or contraction (except for minor adjustments around the edges of the maria), enabling limits of ± 1 km to be applied to the change in lunar radius over the past 4,000 Myr⁶³. This lack of change rules out current claims for massive increases in Earth radius⁶⁴ and restricts the rate of change of G ($-\delta G/G \text{ yr}^{-1}$) to $\leq 5 \times 10^{-11}$ in constant mass cosmologies, and to $\leq 1.5 \times 10^{-10}$ in variable mass cosmologies⁶⁵.

Lunar composition

The overall composition of the Moon is of particular interest because of its possible relationship to the Earth. The highly fractionated nature of the lunar samples to which we have access place constraints on all models. The heat flow⁶⁶ measured at the Apollo 15 and 17 sites is consistent, for a steady-state model, with bulk lunar uranium contents in the range 35–46 p.p.b. A non-steady-state solution could reduce these values by about 20% (ref. 67). A bulk lunar U content of about 40 p.p.b. is estimated from geochemical balance considerations⁶⁸. From correlations among refractory elements (Ca/Al/Ti/U ratios), from K/U systematics (K/U = 2,500) and from the lunar density (3.34 g cm^{-3}) which constrains the Fe content, it is possible to arrive at compositions which satisfy the density, moment of inertia and heat flow constraints. An average of such compositions is given in Table 1. Melting of the outer half or two-thirds of the Moon of such composition gives rise to the feldspathic lunar crust³⁵ as well as a zoned interior capable of producing the mare basalts by partial melting. The depth of initial lunar melting^{57,63} is most probably 300–500 km. If the seismic discontinuity^{2,3} at 480 km is identified with the base of the melted zone, then the seismic velocity decrease (Fig. 1) and the coefficient of moment of inertia value, are consistent with a change to a primitive unfractionated iron-rich interior. The lower portions of the melted zone will be dominated by Mg-rich olivine and orthopyroxene so that a sharp break in seismic velocities is expected. In this model, partial melting in the deep

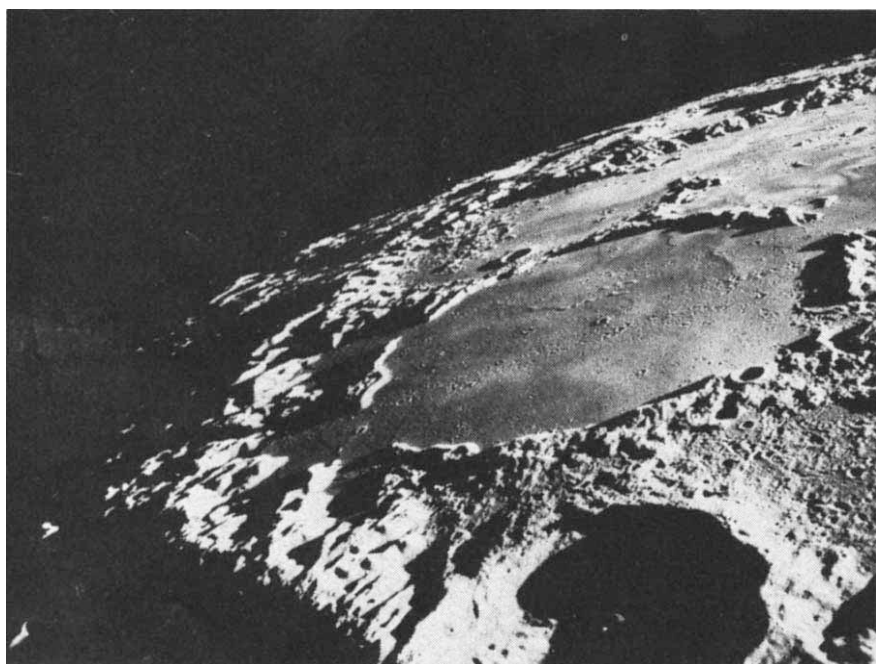


Fig. 4 Farside lunar maria and highlands. The large circular crater, filled with mare basalt, is Thomson (112 km diameter) in the northeast sector of Mare Ingenii (370 km diameter, 34° S, 164°). The large crater in the right foreground is Zelinskiy (54 km diameter). The stratigraphic sequence, from oldest to youngest, is (1) formation of highland crust, (2) excavation of Ingenii basin, (3) formation of Thomson crater, (4) formation of Zelinskiy, (5) flooding of Ingenii basin and Thomson crater with mare basalt, (6) production of small craters on mare surface including a probable chain of secondary craters (NASA AS15-87-11724).

interior is caused by the primitive component of K, U and Th. **Tektites.** The disproving of the hypothesis of the lunar origin of tektites must be counted among the minor successes of the Apollo missions. Recent attempts to revive the hypothesis that they are derived from young lunar volcanoes⁶⁹ encounters similar problems (for example, one to two orders of magnitude age discrepancy, chemical and isotopic distinctions, lack of observed Tertiary lunar volcanoes) to those which ruled out an origin by meteorite splash from the Moon⁷⁰.

Lunar origins

The earlier debate over homogeneous versus heterogeneous accretion of the Moon was resolved in favour of the former hypothesis^{30,40}. Homogeneous accretion of a refractory-rich, and volatile and siderophile element-poor Moon, was followed by extensive melting. The evolution of the Moon is thus well understood, but the pre-accretion scenarios remain indistinct. The three principal pre-Apollo hypotheses all encountered difficulties when confronted with the new data.

Little progress has been made with simple versions of the double planet theory but the oxygen isotope data, perhaps the most significant single cosmochemical observation in the past few years, indicate a close association between the Earth and the Moon⁷³. The depletion in the refractory siderophile elements forbids direct accretion from the solar nebula, so that one or more stages of element fractionation are needed between condensation of the nebula and accretion of the Moon. The location of these events is obscure.

Capture of an already formed Moon has a *deus ex machina* flavour, and has been deemed 'horrendously improbable' (ref. 74). Such an origin, in a different part of the solar nebula, could be predicted to produce some distinct chemical or isotopic signature. In this context, the oxygen isotope data⁷³ may have dealt a fatal blow to the capture hypothesis, by requiring formation of the Earth and Moon in close association. Nevertheless, the presence of fractionated meteorites (enstatite achondrites and aubrites) with similar ¹⁶O enrichments, and the close similarity of the howardites and eucrites to the terrestrial values must engender caution, as more efficient mixing may occur in planetary size bodies. Acquisition of oxygen isotope data from other planets should be accorded a high priority, along with age dating, for future sample return missions.

Fission from the Earth has been extensively debated, because in principle, an evaluation of the theory can be achieved by comparison of lunar compositions with that of the terrestrial mantle. This task is in fact complex, partly due to our lack of understanding of the lateral and vertical variations in the composition of the terrestrial mantle (for an extensive recent review see ref. 75). The siderophile element abundances are particularly crucial, but the reasons for the high abundances of such elements (for example, Ni) in the upper mantle of the Earth remain obscure. It is clear that the terrestrial upper mantle has not been in equilibrium with the core of the Earth⁷². Possibly, the high abundances of elements such as Ni are derived from a post-accretion meteoritic bombardment of the Earth⁷⁶⁻⁷⁸ analogous to such early events on the Moon, Mars and Mercury.

Whatever the cause of the terrestrial siderophile element abundance patterns, the concentrations of refractory siderophile elements Os, Ir, Ni, Pd and Re are enriched by large factors in the Earth compared with the Moon⁷⁹. Au, Sb and Ge are depleted by factors of about 100 in the Moon while the very volatile elements (Bi, Tl, In) show rather uniform depletions of about 50 in the Moon⁷⁹. The alkali elements K, Rb and Cs similarly show depletions by factors of about 2 between the Moon and the mantle of the Earth (Table 1). All these comparisons are complicated by the differing evolutionary histories of the Earth's mantle and the Moon. Simple comparisons, for example, of basalts from both bodies, encounter this problem. Elements such as K and U whose bulk composition can be estimated from a variety of techniques (K-Ar systematics, K/Rb/Sr, Ca/Al/U ratios and heat-flow

Table 1 Composition of the lunar highlands, the bulk Moon and the Earth's mantle

	1	2	3	4
SiO ₂ %	45	42	46.1	45
TiO ₂ %	0.56	0.4	0.2	0.16
Al ₂ O ₃ %	24.6	8.0	4.3	3.3
FeO%	6.6	12	8.2	8.0
MgO%	8.6	31	37.6	40
CaO%	14.2	6.0	3.1	2.6
Na ₂ O%	0.45	0.1	0.4	0.2
K (p.p.m.)	600	80-100	250	150
U (p.p.b.)	240	30-40	—	15
Th (p.p.b.)	900	120-150	—	60
K/U	2,500	2,500	—	10,000

1, Lunar highlands surface⁴⁰; 2, bulk Moon⁷¹; 3, Earth's mantle⁷²; 4, Earth's mantle⁷¹ (NiO = 0.26, Cr₂O₃ = 0.44). p.p.b. = parts per 10⁹.

values) are useful in this context⁷¹. Terrestrial bulk U values of 15-20 p.p.b. are a factor of 2 lower than the lunar abundances (Table 1). Similar constraints apply to the refractory elements Ca, Al and Ti. The Moon also contains more Fe and less Mg than the Earth's mantle (Table 1).

Thus, the weight of the chemical evidence seems to indicate that the terrestrial mantle and the Moon differ substantially in composition for many elements, refractory, volatile and siderophile. The similarity in chemistry between the eucrites and the lunar basalts indicates the existence of similar processes elsewhere in the inner Solar System^{80,81}. The recent demonstration⁸² that the siderophile and volatile element contents of the shergottite meteorites mimic those of terrestrial basalts weakens the case for fission. Thus, the initial attraction of the fission hypothesis (the density similarity between the Moon and the terrestrial mantle) is diminished. Accordingly, very complex scenarios have to be devised in order to derive the Moon from the mantle of the Earth⁸³, or for forming the Moon in close association with the Earth^{75,84,85}. The formation of the Moon from a ring of differentiated debris in terrestrial orbit^{86,87} during the accretion of the Earth accords most closely with the oxygen isotopic evidence⁷³.

Epilogue

Could our insights into the composition, age and evolution of the Moon have been obtained from unmanned missions? The answer is no. Such missions would have returned lunar soils. The formation of these soils was not well understood before the Apollo missions. The unravelling of the age, isotopic and chemical evidence which they contain was only possible because individual components (the hand-collected whole rock samples) of these complex mixtures were also available. Our experience gained from the Apollo missions enables us to understand and extract chemical and age information from such samples⁴⁷, so that now the scientific examination of an unmanned sample collected and returned from Mars or Mercury could be approached with confidence.

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articles

Suspended sediment sources identified by magnetic measurements

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A procedure for identifying the main source of the suspended sediment load of a stream by simple, cheap, rapid and nondestructive magnetic measurements is applicable to samples taken during the routine sampling of flood events permitting general characterisation of the load and more detailed investigation of variations in sediment source both during and between individual flood events.

INFORMATION on the sources of the suspended sediment loads of rivers, and more particularly the relative importance of sheet and rill erosion and channel scour, has many potential uses in studies of sediment transport and sediment yield. For example, it could provide the geomorphologist with a means of elucidating patterns and rates of erosion and afford a means of

highlighting potential problems of nonpoint pollution connected with sediment-associated nutrients and contaminants derived from agricultural land. Existing techniques for determining the relative magnitude of these sources have involved detailed documentation of source area erosion¹ and comparison of measured sediment loads with estimates of sediment yield from sheet erosion derived using a soil loss equation coupled with a sediment delivery term². The first approach involves many operational problems and is essentially impossible for anything except small drainage basins, whilst the latter introduces many uncertainties related to the accuracy of the techniques used to estimate sheet erosion and sediment delivery. Furthermore, both approaches provide only long-term aggregate values and do not permit the study and comparison of individual events. Scope clearly exists for alternative procedures and the use of the properties of the sediment load itself as a means of 'fingerprinting' source areas has considerable promise.

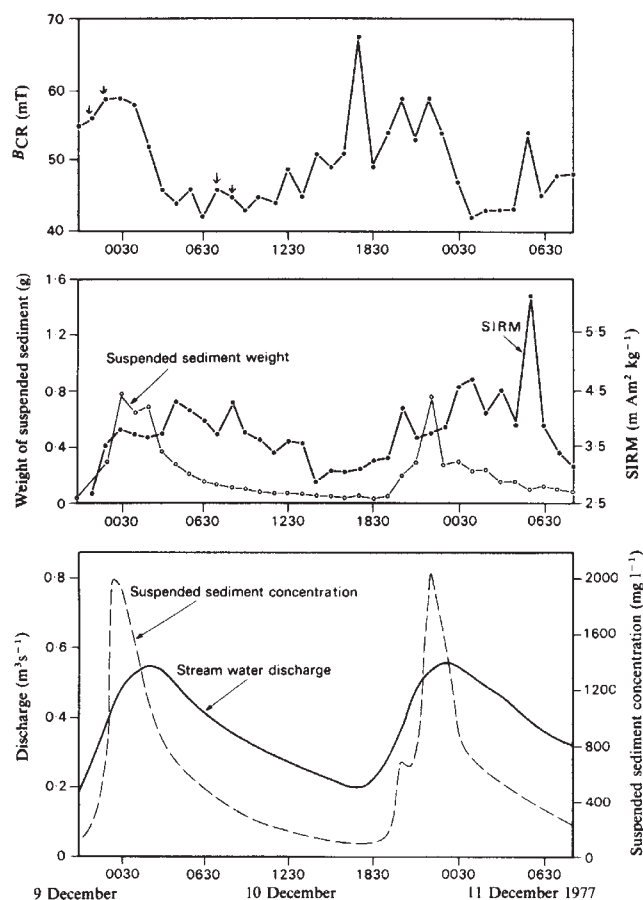


Fig. 1 Variations in B_{CR} , SIRM and suspended sediment concentration measured during a double peaked hydrograph on the Jackmoor Brook. The four samples indexed on the B_{CR} plot are those used to produce the coercivity profiles presented in Fig. 2.

Measurements at Jackmoor Brook

Oldfield *et al.*³ have previously given preliminary results of an attempt to identify the dominant source of the suspended sediment yield from the Jackmoor Brook basin, an instrumented catchment in south-west England, by comparing the magnetic properties of material from potential source areas within the catchment with those of samples of sediment in transit. Their results showed that bulk magnetic susceptibility (χ), saturation isothermal remanent magnetisation (SIRM), SIRM/ χ ratios and coercivity of SIRM (B_{CR}) profiles (Table 1) provide an exceptionally rapid and sensitive basis for differentiating undisturbed woodland topsoil, arable soil, and streambank erosion scars cut into the unmodified Permian bedrock. The processes controlling the transformation of iron compounds which make this type of differentiation possible are poorly understood⁴ but in this case they give rise to an alteration of the fine grained weakly ferromagnetic haematite cement in the unweathered parent material to a ferrimagnetic form during soil development. Fire often causes this transformation, although other less dramatic pedogenic processes may also be responsible. The resulting cubic oxide has been identified as either maghemite⁵⁻⁷ or magnetite⁸. Most evidence suggests that it is impure as a result of isomorphic substitution of iron by other soil cations such as aluminium.

All the magnetic properties previously noted are in some measure diagnostic of this transformation and they permit crude magnetic 'fingerprinting' of different types of sediment source. Oldfield *et al.*³ showed that the magnetic parameters of the four bulk suspended sediment samples measured were closely comparable to those of cultivated topsoil, but that they differed

significantly in several ways from either undisturbed woodland soil or unaltered parent material (Table 1). This suggested that the bulk of the suspended sediment transported by the stream was generated by splash erosion and surface runoff from the extensive cultivated areas of the catchment rather than by channel scour and by erosion of bankside scars. Minor differences in B_{CR} and in SIRM/ χ ratios between the individual suspended sediment samples seemed to be related to the discharge conditions prevailing at the time of sampling and the characteristics of the associated storm runoff event. These differences prompted us to look in more detail at variations in the magnetic properties of the suspended sediment transported by this stream both during and between individual storm events.

Transport of suspended sediment

In the previous study, samples of suspended sediment in transit used for magnetic determinations had been obtained by centrifuging bulk samples of stream water (>20 l) and drying the residual solids. More detailed work on individual storm runoff events necessitated a larger number of samples and in view of the operational problems involved in collecting and transporting numerous bulk samples of stream water and the time consuming nature of the laboratory centrifugation procedure, an alternative means of assembling sediment samples for magnetic measurements was sought. It was found that similar measurements could be undertaken on the residue retained on a glass fibre filter circle after filtration of a relatively small volume of river water (~500 ml). The measurements were unaffected by the presence of the filter medium carrying the sediment. Furthermore the samples collected by automatic pump sampling equipment installed at the outflow gauging station of the Jackmoor Brook catchment could be used for this purpose. This equipment, developed from an earlier design⁹, collects discrete samples at regular six-hourly intervals and is also triggered to collect samples at hourly intervals when the water stage rises above a preset threshold during storm events. The samples have

Table 1 Summary of selected magnetic properties of bulk suspended sediment samples and potential sediment sources from the Jackmoor Brook catchment

Material	χ^* ($\text{mm}^3 \text{kg}^{-1}$)	SIRM [†] ($\text{mA m}^2 \text{kg}^{-1}$)	Property SIRM/ χ [‡] (kA m^{-1})	B_{CR} [§] (mT)	S
Woodland topsoil	>2.5	>10	≈4	>20	1
Cultivated topsoil	0.2–2	1–10	5–7	24–41	0.28–0.8
Poorly drained and gleyed soils	0.06–0.4	0.5–3.5	≈10	≈30	≈0.4
Parent material	<0.1	1–2	>10	200–400	–0.8––0.6
Suspended sediment	0.25–0.75	2.5–9	≈10	37–60	0.06–0.4

* Specific magnetic susceptibility. A measure of the magnetisability of a specimen. Measured in an alternating field of about 0.1 mT peak strength with a frequency of 10 kHz. Mainly reflects the concentration of ferrimagnetic minerals in a specimen.

† Saturation isothermal remanent magnetisation. The magnetisation of a specimen remaining after its removal from a strong (1 T) magnetic field. Mainly reflects the concentration of magnetic minerals in a specimen, but also influenced by the size and shape of magnetic grains.

‡ This ratio reflects the type, size and shape of the magnetic minerals present. For example, it will be decreased by an increasing proportion of ultrafine (superparamagnetic) magnetic grains.

§ Coercivity of remanence. A measure of the stability of the SIRM, being the reverse field required to reduce the remanent magnetisation to zero after saturation in a forward field. Reflects the magnetic mineral type, size or shape, not influenced by concentration.

|| This ratio of the remanence grown in a back field of 100 mT to the SIRM, mainly depends on the ratio of haematite to magnetite minerals in the specimen. A high S value indicates a relatively greater proportion of magnetite in the sample.

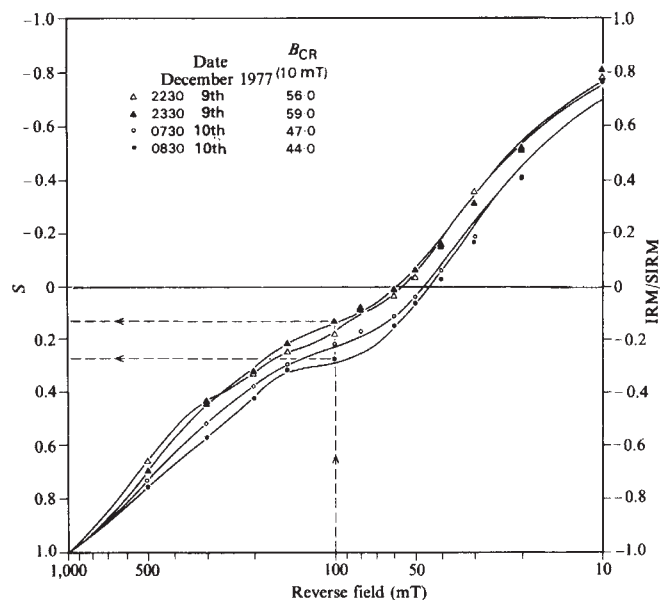


Fig. 2 Coercivity profiles obtained from filter paper residues associated with four samples collected during the storm shown on Fig. 1. Values of S for these samples range from 0.135 to 0.271 and are consistent with the dominance of a cultivated top soil source (Table 1).

been found to be representative of the ambient suspended sediment concentrations at the moment of sampling and may be related to the continuous records of water discharge and suspended sediment concentration (turbidity) available for the gauging site. Samples were therefore collected at frequent intervals during a number of storm events occurring between December 1977 and March 1978.

Samples collected by the automatic sampling equipment were returned to the laboratory for vacuum filtration through pre-weighed glass fibre filter circles (Whatman GFC). Subsequent drying and weighing provided information on the weight of sediment carried by individual filter circles and these were finally moistened, folded, and inserted into polystyrene sample holders with flexible airtight lids.

The SIRM of all resulting samples was determined by placing each one in a 'saturating field' of 1 T and measuring the induced remanent magnetisation (IRM) on a computerised fluxgate magnetometer¹⁰. The noise level of this instrument was two orders of magnitude below the signal from the least magnetic of the samples measured. The signal from the holder and filter medium was minimised by washing every container and lid in distilled water, by handling samples with clean hands and by keeping both empty and full holders in clean sealed polythene bags. Measurements giving values less than four times the mean signal from empty holders containing clean filter circles were rejected. The consistency of the remaining results encourages confidence in measurements made on samples with dry weights as low as 0.02 g (that is well over 90% of the sample analysed). The SIRM measurements, which are specific to unit mass and therefore independent of sample weight, have been completed for over 160 samples covering discharge conditions ranging from 0.05 to $1.0 \text{ m}^3 \text{ s}^{-1}$ and sediment concentrations ranging from 50 to $2,500 \text{ mg l}^{-1}$. Suites of hourly samples spanning five distinct flood events have been analysed.

For the first suite of samples covering the period 2130 h on 9 December 1977 to 0830 h on 11 December 1977, coercivity of SIRM profiles were also obtained for all of the 36 samples. Figure 1 plots the hourly values of SIRM, B_{CR} , and sediment weight along with the record of discharge and suspended sediment concentration for the event. Coercivity profiles were calculated from measurements of remanence in 10–12 reverse fields of increasing strength. Figure 2 shows representative coercivity profiles drawn automatically from the measurements using a smoothing cubic spline function.

For subsequent sample suites, coercivity measurements were made only on extreme samples for which the SIRM values fell close to the outside limits spanned by the first suite. It was also possible to determine magnetic susceptibility for a proportion of the samples with values above the level of repeatable measurement ($1 \times 10^{-7} \text{ m}^3 \text{ kg}^{-1}$) and calculate their SIRM/ χ ratios.

The data on the magnetic properties of suspended sediment transported by Jackmoor Brook, assembled using the filter paper technique described above enable us to evaluate variations in these properties both within and between individual events in terms of the sediment sources and erosion processes involved.

Discussion

Figure 1 illustrates the variations in B_{CR} and SIRM measured during a double peaked hydrograph on the Jackmoor Brook. The variations exhibited are typical of most storm hydrographs and indicate a clear tendency for B_{CR} values to decrease during times of increased runoff and sediment concentration and for SIRM values to increase. The range of values shown are very

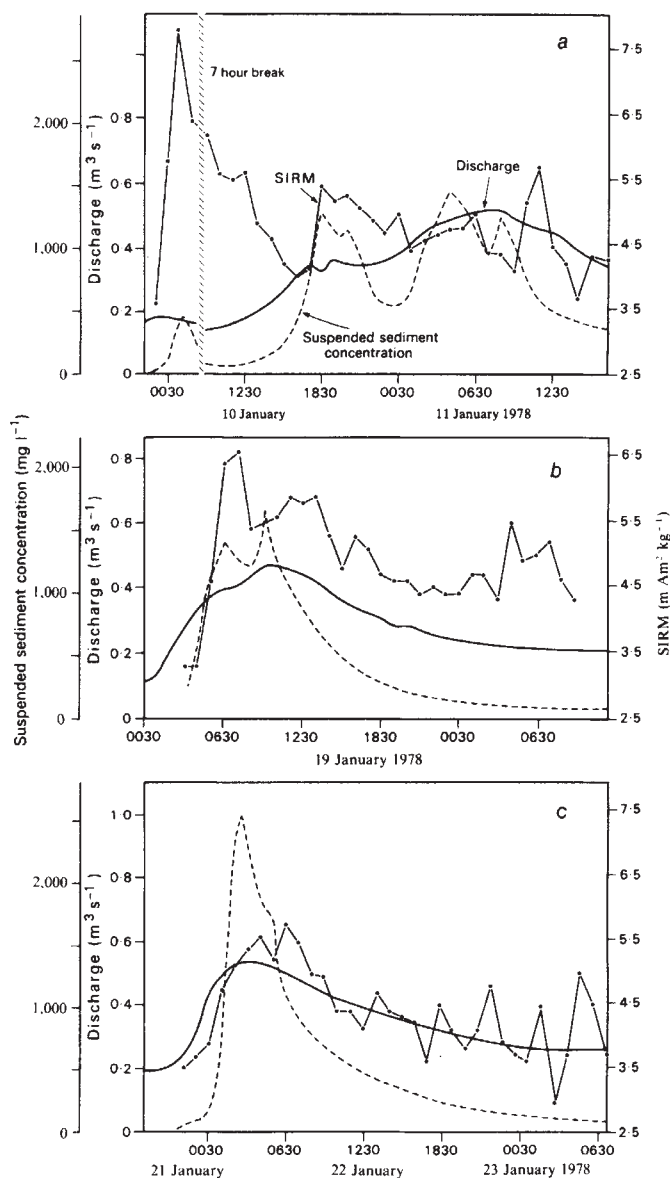


Fig. 3 Variations in SIRM and suspended sediment concentration measured during three storm runoff events on the Jackmoor Brook.

similar to those obtained in the earlier study which suggested that cultivated soils provide the major source of sediment within this catchment (Table 1). Decreasing B_{CR} values and increasing SIRM values indicate a tendency for such sources to become more dominant at the time of the hydrograph peak, because arable soils are marked by higher SIRM and lower B_{CR} values than are the channel-side erosion scars (Table 1). This pattern of variation during the storm hydrograph is therefore consistent with a situation where maximum sediment yields from cultivated areas would coincide with maximum rainfall intensities, maximum surface runoff rates, a maximum area contributing to surface runoff, and therefore the hydrograph peak. Although there is a tendency for the maximum sediment concentrations to precede the discharge peaks in Fig. 1, the maximum SIRM and minimum B_{CR} values occur after the hydrograph peak. This lag effect is again consistent with the sediment sources and process mechanisms that could be postulated for the catchment. Some channel scour must occur and the maximum contribution from this source would be expected to occur during the rising stage of the hydrograph and at the hydrograph peak in response to control by sediment availability and the erosive energy of the streamflow. Many workers¹¹ have noted that sediment contributions from bank erosion are closely controlled by the loosening of sediment particles during preceding dry periods and that the supply from this source is rapidly depleted. The magnetic properties of the suspended sediment would therefore be most influenced by bank contributions during the earlier part of the streamflow hydrograph and this would delay the occurrence of maximum SIRM and minimum B_{CR} values until after the peak flow had passed. It is tempting to attribute the slight reversal in the trend of increasing SIRM values associated with both hydrograph peaks (0030–0230 and 2030–2330 h on 12 December) to a maximum contribution from channel scour at this time but a more detailed sampling programme would be necessary to confirm the precise nature of this reversal. In addition, the higher SIRM values exhibited by the second hydrograph peak are readily explicable in terms of the depletion of channel sources during the first peak and therefore a lesser contribution from channel scour.

Comparison of a number of individual sediment yield events is also facilitated by the ease with which data from filter paper measurements may be collected and Fig. 3 presents the values of SIRM measured during three storm events which succeeded that documented in Fig. 1. Considerable contrasts in levels of SIRM are apparent between these events but the contrasts are again consistent with a simple process-based conceptual model of the variations in the relative importance of slope and channel sources to the sediment budget of this catchment. The full range of SIRM values (2.8–8 mA m² kg⁻¹) indicate that agricultural soils provide the dominant source on all occasions (see Table 1) but relatively low values may be interpreted in terms of proportionally greater contributions from channel scour. The event shown in Fig. 1 was the first major hydrograph of the winter period and might be expected to reflect a relatively high contribution from this source and therefore lower SIRM values. Sediment particles loosened during the preceding dry period would provide a ready supply to channel scour. This source would, however, be rapidly depleted and the higher SIRM values obtained for the two subsequent events (Fig. 3a and b) may be explained both in terms of lack of sufficient time for the supply to be restored and lower discharges which are less effective for channel scour.

The hydrograph which occurred on 22 January 1978 (Fig. 3c) exhibits lower SIRM values than the preceding two events and this feature may be ascribed to the higher discharges involved and therefore to increased channel scour. Higher discharges involve both higher velocities and shear stresses and also submerge greater areas of the channel margin. However, although the discharge peak for this event is of similar magnitude to that of the hydrograph depicted in Fig. 1, the amount of channel scour could be expected to be less than occurred during the first flood event of the winter in view of the depletion effect discussed

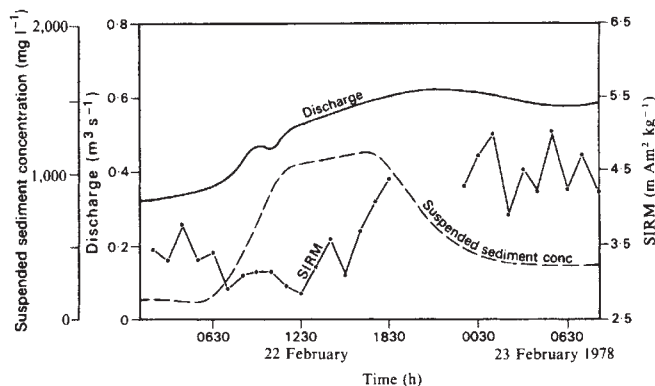


Fig. 4 Variations in SIRM and suspended sediment concentration measured during a snowmelt runoff event on the Jackmoor Brook.

above and this is confirmed by a comparison of the SIRM values. The very high values of SIRM (up to 8 mA m² kg⁻¹) associated with the early part of the storm which occurred on 10 January 1978 (Fig. 3a) are also worthy of comment. In this instance the increase in sediment concentration is not related to a significant increase in discharge and it is unlikely that channel scour could be invoked as the source. A localised input from a field source seems more likely and this is consistent with the high SIRM values which may be viewed as being the result of a lack of dilution by sediment from channel sources. Low values of B_{CR} were also evident during this event.

A final example of variation in the magnetic properties of sediment between individual events is given by Fig. 4. This shows a hydrograph which was unusual in having been produced by snowmelt rather than rainfall. The relatively low suspended sediment concentrations may be explained by the lack of rainfall energy, an important component of splash erosion. SIRM values are also somewhat low and this is consistent with a situation uncondusive to slope erosion and where channel scour would be of increased relative importance compared to other events.

Conclusions

Measurements of the magnetic properties of suspended sediment in transit can provide a general indication of the relative importance of slope and channel sources to the sediment yield of a catchment, and more specific information on the relative contributions of the individual sources during a storm event and on contrasts in the pattern of sediment generation between particular events. The use of filter paper residues for magnetic determinations provides a simple, effective means of collecting the detailed data required for studies of inter- and intra-storm variations. More work is required on sediment enrichment ratios, on the relationship between magnetic properties and the particle size and organic matter content of suspended sediment, and within catchments draining other rock types, if the potential for using magnetic determinations in studies of sediment sources is to be fully realised.

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Fundamental difference between the molecular interactions of agonists and antagonists with the β -adrenergic receptor

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Antagonist binding to the β -adrenergic receptor is largely entropy driven, with only a small enthalpy component. The binding of agonists, on the other hand, is associated with a large decrease in enthalpy which permits a highly unfavourable decrease in entropy. The thermodynamic differences between the binding of agonists and antagonists may provide new insights into the molecular basis for hormone stimulation of adenylate cyclase activity.

THE binding of compounds to the β -adrenergic receptor has been examined in great detail with the use of high-affinity radiolabelled β -adrenergic ligands such as ^{125}I -iodohydroxybenzylpindolol (IHYP)¹⁻³. These studies have increased our understanding of the first step in the activation of adenylate cyclase by catecholamines, but have provided little insight into how binding effects an increase in enzyme activity. It is likely that agonists induce a change in the conformation of the receptor and that this altered form of the receptor interacts, directly or indirectly, with the enzyme to cause activation. Thus, an agonist-induced increase in the apparent Stokes radius of the digitonin-solubilised β -adrenergic receptor has been reported⁴ and it has been shown that agonists cause an increase in the sensitivity of the β -adrenergic receptor to *N*-ethylmaleimide⁵. These results are consistent with the idea that conformational changes occur, but they provide relatively little information about the molecular mechanisms involved.

Differential effects of agonists and antagonists on receptor conformation should be readily apparent in the thermodynamics of the binding reaction. In fact, agonist but not antagonist affinities for β -adrenergic receptors increase at lower temperatures^{6,7}. There is also indirect physiological evidence that agonist affinities are higher at lower temperatures, whereas antagonist affinities are unaffected by changes in temperature⁸⁻¹¹. We report here that the thermodynamics of agonist and antagonist interactions with the β -adrenergic receptor of turkey erythrocytes are substantially different. This difference apparently reflects specific agonist-induced changes in receptor conformation.

Effect of temperature on ligand binding to the β -adrenergic receptor

The turkey erythrocyte membrane is a widely used model for the β -adrenergic receptor/adenylate cyclase system^{1,4-6}. It is a convenient system in which to examine the effects of temperature, as the association and dissociation of IHYP binding to turkey erythrocyte β -adrenergic receptors occur 6–10 times faster than with other β -adrenergic receptors¹². Incubation times required to reach equilibrium are therefore much shorter in the turkey erythrocyte membrane. The effect of temperature on the inhibition of IHYP binding by (–)propranolol and (–)isoprenaline are shown in Fig. 1. The displacement curve of the agonist isoprenaline shifts to the left at lower temperatures, reflecting an increase in affinity, whereas there is little change in the apparent affinity of propranolol. A similar increase in the apparent affinity of isoprenaline with decreasing temperature was also observed in studies of isoprenaline-stimulated adenylate cyclase activity (Fig. 2).

The changes in affinity observed are not a reflection of changes in pH as the K_D values of propranolol and isoprenaline did not change significantly between pH 6.8 and 7.8. The changes were also not an artefact of the longer incubation time used at lower temperatures, as the affinity of the receptor for isoprenaline measured at 37 °C after 5 h at 1 °C was the same as its affinity determined after only the 37 °C incubation. The K_D for isoprenaline at 1 °C was also unchanged by prior incubation for 15 min at 37 °C. The observed changes in affinity are therefore reversible and apparently a function of temperature alone. Full agonists displayed the largest increases in affinity with a lowering of temperature whereas the affinities of antagonists were essentially unchanged (Table 1). The effect of temperature on partial agonists was less than that on full agonists.

Thermodynamic analysis of the temperature dependence of binding

The standard Gibbs free energy change (ΔG°) of binding (association) was calculated from the equation

$$\Delta G^\circ = -RT \ln K_A \quad (1)$$

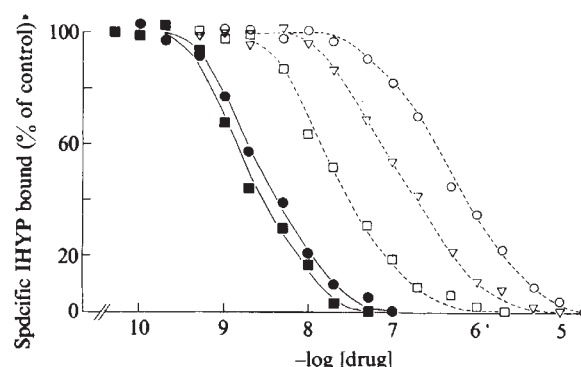


Fig. 1 Effect of temperature on the inhibition of specifically bound IHYP by (–)isoprenaline and (–)propranolol. Turkey erythrocyte membranes were prepared as described by Øye and Sutherland³³ and stored at –70 °C in 0.145 M NaCl containing 10 mM Tris-Cl (pH 7.4) and 1 mM EDTA at a protein concentration of 40 mg ml^{–1}. Immediately before use the membranes were thawed and diluted 100-fold in ice-cold isotonic saline containing 20 mM Tris-Cl (pH 7.5). Following centrifugation at 20,000g for 10 min at 4 °C, the membranes were resuspended in buffered saline to a protein concentration of 0.1 mg ml^{–1}. ^{125}I -iodohydroxybenzylpindolol (IHYP, 2.2 Ci μmol^{-1}) was prepared and purified as previously described^{2,3}. Turkey erythrocyte membranes (10 μg protein) were incubated with IHYP (45,000–55,000 c.p.m.) and competing ligand ((–)isoprenaline: $\circ$, ∇ , $\square$; (–)propranolol: $\bullet$, $\blacksquare$) in 0.25 ml of 0.09 M NaCl containing 1.1 mM ascorbic acid and 12 mM Tris-Cl (pH 7.2 at 24 °C) for 15 min at 37 °C ($\circ$, $\bullet$), 2.5 h at 10 °C (∇), and 5 h at 1 °C ($\square$, $\blacksquare$). Bound IHYP was determined by filtration through glass fibre filters as previously described³⁴. GTP (100 μM) had no effect on either agonist or antagonist binding at 1 °C or 37 °C. Specific binding was defined as the amount of IHYP bound in the absence of competing ligand minus the amount bound in the presence of 30 μM (–)isoprenaline. Specific binding routinely represented 90% of the total IHYP bound. Each point represents the mean of three experiments done in duplicate.

Table 1 Equilibrium dissociation constants of β -adrenergic ligands at 37 °C and 1 °C

	K_D (nM)		$\frac{K_D(37^\circ\text{C})}{K_D(1^\circ\text{C})}$
	37 °C	1 °C	
Agonists			
(-)isoprenaline	254 ± 18	11 ± 2.0	23.3
(-)noradrenaline	2,680 ± 280	48 ± 1.5	55.5
(-)adrenaline	5,230 ± 960	326 ± 95	16.0
Partial agonists			
Soterenol	1,620 ± 70	305 ± 51	5.3
Fenoterol	3,160 ± 250	868 ± 56	3.6
Salmefamol	7,460 ± 460	1,130 ± 80	6.6
Metaproterenol	16,800 ± 2,200	1,670 ± 510	10.1
Terbutaline	43,900 ± 7,200	18,200 ± 1,300	2.4
Antagonists			
(-)propranolol	1.6 ± 0.21	0.59 ± 0.032	2.6
MK-950	2.1 ± 0.18	2.5 ± 1.06	0.84
IPS-339	2.2 ± 0.43	2.3 ± 0.49	0.95
Pindolol	4.5 ± 0.12	1.5 ± 0.39	2.95
Zinterol	372 ± 29	196 ± 19	1.92
Metoprolol	1,300 ± 180	1,130 ± 20	1.15
Sotatol	1,660 ± 90	1,050 ± 150	1.58
H 35/25	2,510 ± 440	1,700 ± 100	1.48
Butoxamine	3,760 ± 440	2,990 ± 84	1.26
OPC 2009	4,810 ± 250	3,970 ± 330	1.21
Atenolol	5,300 ± 90	2,530 ± 510	2.09
Practolol	5,640 ± 520	12,600 ± 4,400	0.45

IC_{50} values were determined from Hill plots of the inhibition of specific IHYP binding at 37 °C and 1 °C (see Fig. 1). K_D values were calculated from the IC_{50} values by the method of Cheng and Prusoff³⁶ using the equation: $K_D = IC_{50}/(1 + L/K_L)$, where L is the concentration of free IHYP and K_L the equilibrium dissociation constant for IHYP determined by Scatchard analysis. Hill coefficients for the binding of ligands did not differ significantly from 1.0. K_L was 42 pM at 37 °C and 20 pM at 1 °C. The number of IHYP binding sites (B_{max}) was 372 $\pm$ 10 fmol per mg protein at 37 °C and 375 $\pm$ 10 fmol per mg protein at 1 °C. The results shown are expressed as the mean $\pm$ s.e.m. for 3–10 determinations done in duplicate.

where R is the gas constant (1.99 cal mol⁻¹ deg⁻¹), T is the temperature in degrees Kelvin, and K_A is the equilibrium association constant ($1/K_D$). Changes in the standard Gibbs free energy reflect changes in enthalpy (ΔH°) as well as changes in entropy (ΔS°) as

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \quad (2)$$

The enthalpy change of the binding reaction was determined using the integrated van't Hoff equation

$$\ln K_A = -\Delta H^\circ/RT + \Delta S^\circ/R \quad (3)$$

The slope of a plot of $\ln K_A$ against $1/T$ is $-\Delta H^\circ/R$. Once ΔG° and ΔH° had been determined, the standard entropy change of the reaction (ΔS°) could be calculated from equation (2).

Van't Hoff plots for the binding reaction of five representative β -adrenergic ligands are shown in Fig. 3. The plots are linear over the temperature range studied, showing that the enthalpy change is independent of temperature. Both the enthalpy and the entropy changes of agonist binding are substantially different from those of the antagonists, despite the overlap in the changes in Gibbs free energy (Table 2). The most striking finding is that binding of full agonists results in a decrease in entropy. This decrease is thermodynamically unfavourable and it is only because of the large decreases in enthalpy that binding can occur. Thus, the binding of agonists is enthalpy driven, with marked net decreases in entropy, whereas the binding of antagonists seems to be almost completely entropy driven.

An excellent correlation was observed when the decreases in enthalpy and the efficacies of full and partial agonists were compared (Fig. 4A). Antagonists have zero efficacy and their binding is associated with an increase in entropy ranging from +13 to +42 entropy units (cal deg⁻¹ mol⁻¹) (Fig. 4B). Full agonists had ΔS° values of less than -12 entropy units, and the binding of partial agonists had intermediate entropy values.

Molecular interpretations of the thermodynamics of binding

The entropy-driven binding of antagonists to the β -receptor is thermodynamically similar to many other protein-binding reactions where information transfer is not involved. Thus, for the interaction of ovalbumin with rabbit anti-ovalbumin antibody, $\Delta H^\circ = 0$ kcal mol⁻¹, and $\Delta S^\circ = +20$ entropy units (EU) (ref. 12), and for the binding of organic ions to bovine serum albumin, $\Delta H^\circ = -2$ kcal mol⁻¹ and $\Delta S^\circ = +14.5$ to +17.1 EU (ref. 13). These reactions are probably driven by the increases in entropy which result when water molecules, ordered around the ligand and the binding site, are displaced¹⁴. In view of the relatively non-polar nature of the β -adrenergic receptor antagonists, it is likely that the binding is largely hydrophobic although other forces, including ionic interactions, hydrogen bonding and Van der Waals interactions, may also be involved. However, net enthalpy changes would not be expected because dissolution of energetically similar bonds with water would necessarily occur. In any case, these types of interaction are also entropy driven^{15,16}.

The thermodynamics of agonist-receptor interactions are substantially different from those of antagonist binding and other passive ligand-protein reactions. Note, however, that the thermodynamic parameters measured are net changes resulting from the interaction of a ligand and protein. These thermodynamic changes can also reflect induced conformational changes in the receptor or in adjoining molecules. As discussed above, binding reactions in general have a large positive entropy component. It is reasonable to suggest that occupation of the receptor by agonists also results in an increase in entropy. The net decrease in entropy observed on agonist binding may reflect

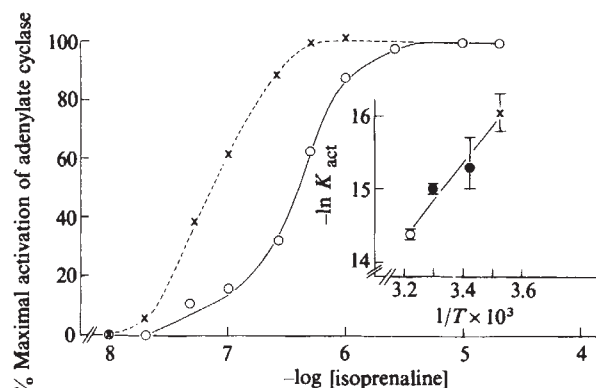


Fig. 2 Effect of temperature on the concentration dependence of (-)-isoprenaline activation of adenylate cyclase. Adenylate cyclase activity was determined by measuring the conversion of [α -³²P]ATP to ³²P-labelled cyclic AMP and isolating the product by a modification of the method of Salomon *et al.*³⁵ as previously described³⁴. The reaction was carried out in a final volume of 0.2 ml including turkey erythrocyte membranes (1–3 mg protein), 50 mM Tris-maleate (pH 7.5 at 24 °C), 5 mM cyclic AMP, 0.75 mM isobutylmethylxanthine, 5 mM MgCl₂, 0.5 mM EGTA, 0.25 mM ATP, 0.1 mg ml⁻¹ creatine kinase, 10 mM creatine phosphate and 30 μ M GTP. Reactions were run for 10 min at 37 °C (○) and for 60 min at 10 °C (×). Each point represents the mean of three experiments done in duplicate. Basal activities (in pmol cyclic AMP formed per min per mg protein) were: 0.55 $\pm$ 0.045 at 37 °C and 0.060 $\pm$ 0.0024 at 10 °C. Maximal per cent stimulation by isoprenaline decreased with decreasing temperature. Isoprenaline stimulated adenylate cyclase 7.8-fold at 37 °C, 3.2-fold at 30 °C, 2.2-fold at 20 °C and 1.3-fold at 10 °C. Adenylate cyclase activity was linear with protein up to at least 4 mg. Inset: Van't Hoff plot (see text) of the dependence of K_{act} of (-)-isoprenaline on temperature. K_{act} is the concentration of (-)-isoprenaline which activates adenylate cyclase to 50% of maximum. Each point represents the mean of three experiments done in duplicate $\pm$ the s.e.m. The correlation coefficient (r^2) was 0.985, calculated by the method of least squares, and the enthalpy change of the reaction (ΔH°) was -9.9 kcal mol⁻¹, calculated from the slope of the line ($-\Delta H^\circ/R$; see text).

Table 2 Thermodynamic parameters of ligand binding to the β -adrenergic receptor of turkey erythrocytes

	ΔG° (kcal mol ⁻¹)	ΔH° (kcal mol ⁻¹)	ΔS° (entropy units)
Agonists			
(-)isoprenaline	-9.39	-13.39	-12.9
(-)noradrenaline	-7.91	-18.86	-35.3
(-)adrenaline	-7.50	-12.75	-16.9
Partial agonists			
Soterenol	-8.23	-7.84	+1.26
Fenoterol	-7.81	-6.06	+5.65
Salmefamol	-7.28	-8.86	-5.10
Metaproterenol	-6.78	-10.83	-13.06
Terbutaline	-6.19	-4.13	+6.65
Antagonists			
(-)propranolol	-12.51	-3.85	+27.9
MK-950	-12.32	+0.81	+42.3
IPS-339	-12.30	+0.25	+40.5
Pindolol	-11.85	-5.08	+21.8
Zinterol	-9.13	-3.06	+19.6
Metoprolol	-8.36	-0.66	+24.8
Sotalol	-8.21	-2.15	+19.5
H 35/25	-7.96	-1.83	+19.8
Butoxamine	-7.71	-1.08	+21.4
OPC 2009	-7.55	-0.90	+21.5
Atenolol	-7.49	-3.47	+13.0
Practolol	-7.46	+3.92	+36.7

Assays were carried out in 0.09 M NaCl containing Tris-Cl (0.012 M, pH = 6.8). The changes in free energy (ΔG°), enthalpy (ΔH°) and entropy (ΔS°) of binding at 37°C were calculated as described in the text.

an induced conformational change of the receptor protein or immediately surrounding membrane components. The net decrease in entropy would be the sum of the increase in entropy associated with binding and the larger decrease resulting from conformational changes. The large net decrease in enthalpy may

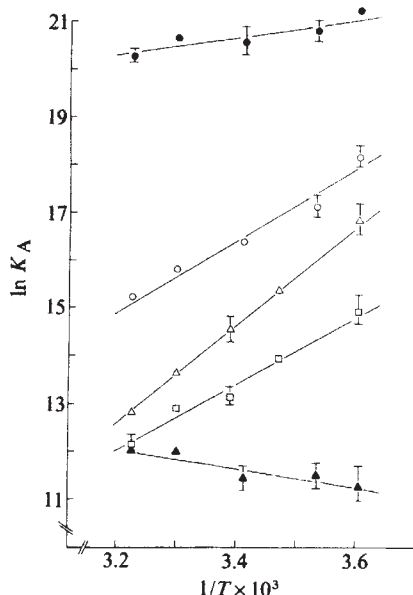


Fig. 3 Van't Hoff plots of the dependence of K_A on temperature. The data shown are for (-)propranolol (●), (-)isoprenaline (○), (-)noradrenaline (Δ), (-)adrenaline (□) and practolol (▲). K_A values were calculated from the equilibrium dissociation constants ($K_A = 1/K_D$) determined as described in Table 1. Each point represents the mean of 3–10 determinations done in duplicate $\pm$ the s.e.m. The s.e.m. of points with no error bars indicated were less than the size of the symbols. The dissociation constants for IHYP (in pM) were: 42 (37°C), 33 (30°C), 27 (24°C), 23 (20°C), 23 (15°C), 22 (10°C) and 20 (1°C). The density of binding sites did not vary with temperature. The slope of the line is equal to $-\Delta H^\circ/R$ (see text).

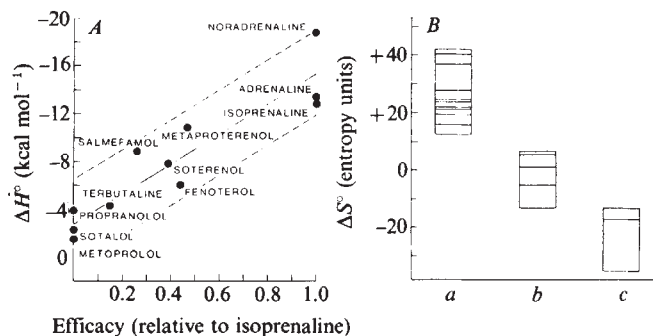


Fig. 4 Relationship between the enthalpy and entropy of binding and efficacy. **A**, The changes in enthalpy for the binding of ligands are plotted as a function of their efficacy. Efficacy was measured as the maximal activation of adenylate cyclase at 37°C and is expressed as activation relative to the maximal activation by 30 μ M (-)isoprenaline. Only three representative antagonists are shown. The solid line represents a linear regression analysis of the data shown. The correlation coefficient is 0.916. The dashed lines show the range of the values observed. **B**, The changes in entropy for the binding of the ligands in Table 2 are plotted as a function of antagonist (a), partial agonist (b) and full agonist (c) properties. Each horizontal line represents a ligand. The average entropy change for each group is: antagonists, $+25.7 \pm 2.7$ EU; partial agonists, -0.92 ± 3.57 EU; full agonists, -21.7 ± 6.19 EU; The mean values of antagonists, partial agonists and full agonists differed from one another ($P < 0.01$). A plot of ΔS° against efficacy (not shown) gave a correlation coefficient of 0.888.

primarily reflect the lower enthalpy of the particular conformational state of the receptor induced by the agonists.

A two-step model similar to that proposed for agonist interactions with the nicotinic receptor^{16,17} may be useful for β -adrenergic receptors.



In this model, L is the ligand, R the receptor and R' the agonist-induced state of the receptor protein. Antagonists would participate only in reaction 1, with little or no change in enthalpy and large increases in entropy. Agonists and antagonists may bind in reaction 1 with similar thermodynamic components; however, because of structural differences agonists are able to induce a conformational change of the receptor to R' . This conversion, reaction 2, would have negative enthalpy and entropy components. A large decrease in enthalpy could compensate for an unfavourable decrease in entropy and give reaction 2 a net negative free energy change, allowing it to occur. The extent of conversion to the R' state, the receptor conformation which leads to activation of adenylate cyclase, would be reflected in the efficacy of the particular ligand. This is consistent with the observed correlation between efficacy and both ΔH° and ΔS° .

General use for the thermodynamic approach

Although the turkey erythrocyte membrane preparation is widely used for the study of the β -adrenergic receptor/adenylate cyclase system, the pharmacological specificity of β -adrenergic receptors on these cells is different from that of mammalian β_1 - or β_2 -adrenergic receptors¹²; there is no effect of GTP on agonist binding¹⁸, and 'desensitisation' does not occur¹⁹. Despite these differences, the results and interpretation discussed above may be generally applicable. Preliminary results have shown differences in the thermodynamics of agonist and antagonist binding to β -adrenergic receptors of rat cerebral cortex and heart similar to those reported here (unpublished results). There is also physiological evidence that β -adrenergic receptors in several tissues have higher affinities for agonists at lower temperatures than at more physiological temperatures^{7–10,20–22}.

Various treatments cause agonist-specific changes in β -adrenergic receptor affinity. As affinity is a function of changes in the enthalpy and entropy of binding, alterations in affinity will reflect changes in these components. Thus, thermodynamic analyses of the agonist-specific changes in affinity which have been reported following cell disruption^{23,24} and membrane solubilisation^{6,25} and treatment with guanine nucleotides^{26,27} or divalent cations²⁸ may contribute to our understanding of the molecular mechanisms involved in these changes.

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Finally, temperature-dependent changes in apparent affinity have also been observed for the α -adrenergic receptor²⁹, histamine receptor^{30,31} and for the affinity of ³H-flunitrazepam for benzodiazepine receptors³². Thus, thermodynamic analysis may be a generally useful tool for examining the molecular events involved in hormone-receptor interactions.

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Chicken ovalbumin contains an internal signal sequence

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Ovalbumin is shown to contain an internal rather than an amino-terminal signal sequence. This internal signal can be recovered in a tryptic fragment which comprises residues 229–276 of mature ovalbumin and contains a region of striking sequence homology to amino-terminal cleaved signals of two other oviduct secretory proteins. The isolated tryptic fragment is used as a probe to demonstrate that a signal receptor is present in membrane vesicles derived from the rough but not in those derived from the smooth endoplasmic reticulum.

THE information for translocation of polypeptides across membranes was proposed¹ to reside in a discrete portion of the polypeptide chain, termed the signal sequence. For many secretory proteins the signal has been shown to consist of a short-lived amino-terminal extension, 15–30 amino acid residues long¹, that is cleaved from the chain during its synthesis and translocation across the rough endoplasmic reticulum (RER) membrane. Although the primary structure of the signals for various secretory proteins differs, there is circumstantial evidence that they all share certain features in their secondary structure¹.

In addition to secretory proteins, certain integral membrane proteins have been shown to contain a signal sequence that is functionally equivalent to that of secretory proteins². These data suggest the existence in the RER of only one polypeptide transport system (translocator) that mediates transfer across the membrane in response to a signal which is common to secretory and certain integral membrane proteins.

It has been anticipated that cleavage of the signal is not an obligatory step for translocation of proteins across the RER membrane³. Ovalbumin seemed to provide a first example as it was found to be synthesised without the typical transient signal sequence⁴. Moreover, data obtained from *in vitro* translocation and competition experiments⁵ provided strong evidence for the existence in *de novo* synthesised ovalbumin of an uncleaved signal sequence that was functionally identical to the cleaved signals of other secretory proteins. Like its cleaved counterpart the uncleaved signal of ovalbumin is expressed only by nascent but not by the completed, presumably folded chain⁵. Consequently, it was reasoned⁵ that mature and secreted ovalbumin should contain a 'latent' signal that might be uncovered after denaturation or limited splitting of the molecule. The data presented here demonstrate that this hypothesis was correct, and, surprisingly, that ovalbumin contains an internal rather than an amino-terminal signal sequence.

Detection of signal activity in mature ovalbumin

To determine signal activity we measured the extent to which ovalbumin or its fragments are able to inhibit competitively *in vitro* translocation of nascent bovine pre-prolactin into dog pancreas microsomal vesicles⁵. The assay system consists of a cell-free protein-synthesising system (in the present case, a nuclease-treated rabbit reticulocyte lysate⁶) programmed with bovine pituitary mRNA and supplemented with ribosome-stripped and nuclease-treated microsomal vesicles from dog pancreas⁷. In the absence of microsomal vesicles, translation of bovine pituitary mRNA yields a major product (Fig. 1a, lane 1), previously shown⁸ to be pre-prolactin (bovine pre-prolactin is

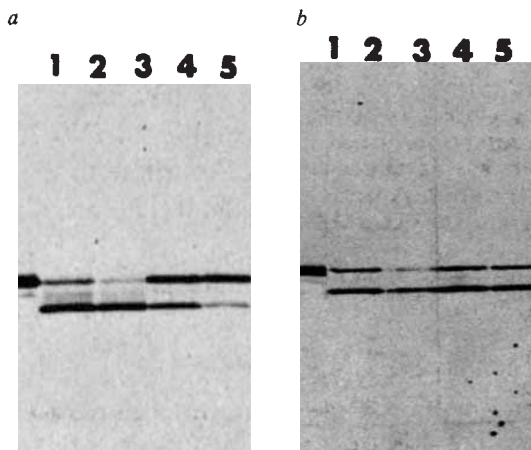


Fig. 1 Effect of unfractionated tryptic ovalbumin or serum albumin fragments on nascent pre-prolactin translocation. *a*, Bovine anterior pituitary mRNA ($0.2 A_{260}$ units ml^{-1}) was translated in a nuclease-treated rabbit reticulocyte lysate containing $250 \mu\text{Ci ml}^{-1}$ of ^{35}S -methionine (650 Ci mmol^{-1}) either in the absence (lane 1) or presence (lanes 2–5) of $1.0 A_{260}$ units ml^{-1} of nuclease-treated dog pancreas microsomal membranes⁷. Incubation mixtures also contained 8 mg ml^{-1} of native ovalbumin (lane 3), 8 mg ml^{-1} of denatured ovalbumin (lane 4) or 0.5 mg ml^{-1} of tryptic ovalbumin fragments (lane 5). Preparation of denatured ovalbumin (OV_d) and tryptic ovalbumin fragments (OV_f) is described in Fig. 2 legend. Preparations of OV_d and OV_f were desalted²⁵ on Sephadex G-25 before being added to the incubation mixture. After incubation for 1 h at 25°C , samples were prepared for polyacrylamide slab gel electrophoresis in SDS⁸. *a*, Shows an autoradiograph of the dried slab gel. Positions of pre-prolactin and prolactin are indicated by upper and lower arrowheads, respectively. *b*, As in *a*, except that equivalent concentrations of bovine serum albumin in native (lane 3), denatured (lane 4) or trypsin-digested (lane 5) forms was substituted for the corresponding ovalbumin forms.

prolactin plus an amino-terminal signal of 30 amino acid residues⁸). In the presence of microsomal membranes, however, prolactin is also synthesised (Fig. 1*a*, lane 2, and ref. 8), in amounts which depend on the concentration of microsomal vesicles: increasing concentrations of vesicles result in an increased synthesis of prolactin relative to pre-prolactin⁸. It has been demonstrated⁸ that prolactin synthesis is the result of translation-coupled translocation of nascent pre-prolactin to the lumen of the microsomal vesicles where the signal is cleaved from the growing uncompleted chain. Following cleavage by signal peptidase on the luminal side of the microsomal vesicle, the nascent prolactin chain continues to grow and to be translocated until it is completed and entirely segregated within the lumen of the microsomal vesicle⁸. This *in vitro* synthesised and segregated prolactin is indistinguishable from mature prolactin⁸. Thus, because of their exclusive location—all pre-prolactin molecules outside the microsomal vesicles (sensitive to proteolysis) and all prolactin molecules inside the vesicles (resistant to proteolysis)—the ratio of prolactin to pre-prolactin is an accurate indicator for the extent of translocation^{7,8}.

As expected⁵, mature native ovalbumin was ineffective as a competitive inhibitor for nascent pre-prolactin translocation, as the ratio of prolactin to pre-prolactin synthesised in the presence of mature native ovalbumin (Fig. 1*a*, lane 3) was comparable to that synthesised in its absence (Fig. 1*a*, lane 2). However, denatured ovalbumin was an inhibitor of nascent pre-prolactin translocation, significantly reducing the ratio of prolactin to pre-prolactin (Fig. 1*a*, lane 4). This inhibitory activity was dependent on the concentration of the added denatured ovalbumin and was lost on renaturation (data not shown).

Tryptic dissection of ovalbumin

The finding that mature ovalbumin molecules contain a latent signal activity that is uncovered on denaturation was of practical importance for our objective of undertaking a limited fragmentation of the ovalbumin molecule. Using this, we hoped to isolate an active fragment and localise the signal activity to a

defined portion of the molecule. It would thus be possible to monitor rapidly the dissection process of the denatured ovalbumin molecule (by comparing its signal activity with that of the generated mixture of fragments) and thereby to establish conditions for its maximal fragmentation without a concomitant loss of signal activity. Such conditions were established by using trypsin as the dissecting tool. The tryptic ovalbumin fragments (see Fig. 2, lane 2) inhibited translocation of nascent pre-prolactin (Fig. 1*a*, lane 5).

Furthermore, neither mature (Fig. 1*b*, lane 3), denatured (lane 4) nor fragmented (lane 5) bovine serum albumin was able to inhibit translocations of nascent pre-prolactin. These results suggested that the inhibition of translocation observed with ovalbumin fragments was not a general property of secretory proteins or a nonspecific effect of hydrophobic peptides released from these globular proteins.

Purification and characterisation of an active ovalbumin fragment (OV_f)

The tryptic digestion mixture was subjected to conventional fractionation procedures described in detail in Fig. 2 legend.

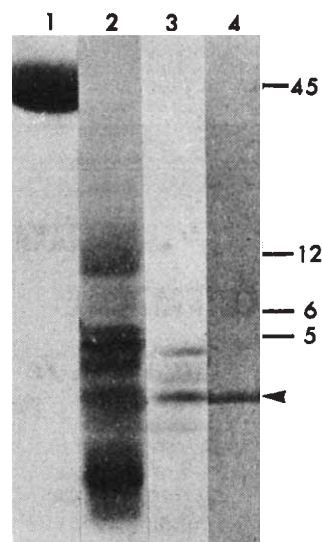


Fig. 2 Purification from trypsinised ovalbumin of a fragment with signal activity, monitored by polyacrylamide gel electrophoresis in SDS and 7 M urea. One gramme of chicken ovalbumin (Worthington) was dissolved in 20 ml of 0.25 M Tris-HCl, pH 7.5; 24 g of urea and 1.2 ml of 1.0 M dithiothreitol (DTT) were added and the solution volume was adjusted with water to 50 ml. After incubation at 37°C for 1 h and at 100°C for 15 min, the solution (containing 'denatured' ovalbumin (OV_d)) was diluted to 100 ml with water. 10 mg of trypsin-tosylphenylethylchloromethyl ketone (Worthington) were added every 6 h during 24-h incubation at 37°C . Proteolysis was terminated by addition of phenylmethylsulphonyl fluoride (PMSF) to a final concentration of 2 mM. This solution of tryptic ovalbumin fragments was designated OV_f . Following isoelectric precipitation at pH 5.0 with glacial acetic acid, the precipitate was dissolved in 100 ml of 50 mM Tris-HCl, pH 7.5, 15 mM NaCl, 1 mM DTT and adjusted to pH 7.5 with 1.0 M NaOH. Ion exchange chromatography on DEAE-cellulose (25 ml bed volume) was carried out with 200 ml of a linear gradient of 15–750 mM NaCl containing 50 mM Tris-HCl, pH 7.5, and 1 mM DTT. Fractions (5 ml) were collected and assayed for signal activity, which was found to elute in a volume of ~40 ml with a peak at 400 mM NaCl. The material in this fraction was concentrated by another cycle of isoelectric precipitation at pH 5.0. The resulting pellet was dissolved in 5 ml of solution A (6 M urea, 50 mM Tris-HCl, pH 7.5, and 10 mM DTT) and applied to a column of Sephadex G-50 fine equilibrated with solution A. A major A_{280} peak eluted like a polypeptide of molecular weight (MW) ~8,000, in a total volume of 30 ml. This material is designated OV_{800} . After dilution with water to 100 ml, an aliquot (1 ml) was subjected to polyacrylamide slab gel electrophoresis in 7 M urea (without SDS). Four distinct bands, similar in intensity and mobility to those shown in lane 3, were observed (data not shown). Individual bands were excised from the unstained gels and eluted by shaking, each into a volume of 5 ml of 5 mM Tris-HCl, pH 7.5. Following lyophilisation, each of the peptides was resuspended in 0.1 ml of 20 mM Tris-HCl, pH 7.5, and tested for signal activity. One of the peptides, designated as OV_f , was found to be active (see Fig. 3). Lane 1, 100 μg denatured ovalbumin (OV_d), note the absence of detectable degradation products; Lane 2, 20 μl of trypsin-digested ovalbumin (OV_f); Lane 3, 20 μl of OV_{800} ; Lane 4, 10 μl of OV_f . Numbers to the right of lane 4 refer to size ($\times 10^3$) of MW markers.

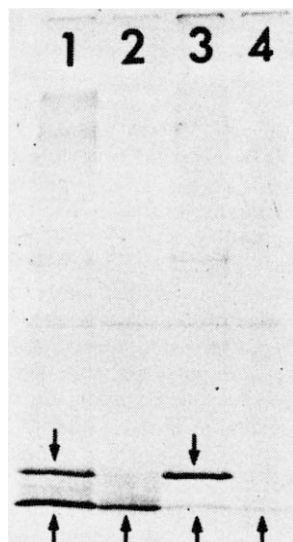


Fig. 3 Inhibition of nascent pre-prolactin translocation by a tryptic ovalbumin fragment (OV_s). Bovine pituitary mRNA was translated in the presence of microsomal membranes as described in Fig. 1 legend. OV_s (see lane 4 of Fig. 2) was either absent (lanes 1 and 2) or included (lanes 3 and 4) in the incubation mixture. Following termination of incubation for protein synthesis, aliquots (lanes 2 and 4) were subjected to post-translational proteolysis (incubation with 200 µg ml⁻¹ each of trypsin and chymotrypsin for 1 h at 0 °C in the presence of 10 mM CaCl₂). Proteolysis was terminated by the addition of Trasylol² and 1 mM PMSF followed by preparation for SDS-polyacrylamide slab gel electrophoresis. The figure shows an autoradiograph of the dried slab gel. Downward pointing arrow indicates pre-prolactin, upward pointing arrow indicates prolactin.

Table 1 Amino acid composition of OV_s compared with that of mature ovalbumin (OV₁₋₃₈₅)⁹ and selected tryptic ovalbumin fragments (OV₂₂₉₋₂₇₆ and OV₁₋₄₆)

Amino acid	OV _s	OV ₂₂₉₋₂₇₆	OV ₁₋₄₆	OV ₁₋₃₈₅
A	1.5	1	7	35
C	0.0	0	2	6
D/N	2.9	3	3	31
E/Q	9.4	9	3	48
F	2.0	2	4	20
G	2.1	2	3	19
H	0.0	0	2	7
I	2.0	3	4	25
K	0.7	1	3	20
L	7.7	8	3	32
M	3.2	3	3	16
P	2.2	2	1	14
R	0.8	1	0	15
S	5.5	6	3	38
T	2.6	3	0	15
V	3.2	3	3	31
W	(+)	1	0	3
Y	0.0	0	2	10

translocation system was due to competition (with the amino-terminal and cleavable signal of nascent pre-prolactin) for a site on the RER translocator; this premise needed to be substantiated to rule out an alternative mode or site of action of OV_s.

Homology of OV_s to signals of other chicken oviduct secretory proteins

Convincing evidence for the notion that OV_s contains the equivalent of the ovalbumin signal was obtained from a structural comparison (Fig. 4) of OV_s with the amino-terminal and cleaved signals of two other hen oviduct secretory proteins, ovomucoid and lysozyme^{10,11}. There is clearly a significant degree of sequence homology. Particularly striking are two clusters of homology, each comprising three hydrophobic residues. There are also clusters of amino acid residues (indicated in bold face, Fig. 4) differing in their genetic code by a single base.

	235	240	245	250
OV _s	F A S G T M S M L V L	- - -	L P D E V S G L E	
pre OVM	F V L - - F S - F V L	C G F L P D	A A F G A E	
pre LYS	M R S - - L L I L V L	C - F L P L A A L G	K V	

Fig. 4 Homology of OV_s to amino terminal cleaved signals of other chicken oviduct secretory proteins. The location of OV_s within the ovalbumin molecule was deduced from its distinctive amino acid composition (see Table 1) and the known⁹ sequence of ovalbumin. Signal sequences^{10,11} of pre-ovomucoid (pre OVM) residues 7-24 and of pre-lysozyme (pre LYS) residues 1-18 were each aligned to maximise homology to ovalbumin residues 234-254 within OV_s. Homologous residues between OV_s and pre OVM or between OV_s and pre LYS are boxed. Residues involving a single base change are indicated in bold face. Cleavage site of pre OVM and pre LYS is after glycine and is aligned with Gly 251 of ovalbumin.

Furthermore, the penultimate Gly residue of the two cleaved signals readily aligns with a Gly residue in OV_s. However, ovalbumin is not cleaved at this site. A possible explanation for this could be the absence of Cys or of Cys-Gly, located between the two hydrophobic homologous clusters in the case of the two cleaved signals but absent in OV_s (Fig. 4). It is likely^{1,12} that this region constitutes the site of a β-turn or another secondary structural feature that is important for recognition by signal peptidase. Consistent with this reasoning is the recent demonstration¹³ that a replacement of a glycyl residue by an aspartyl residue at position 14 of the 20-residue-long signal of lipoprotein in an *Escherichia coli* mutant results in the loss of the option to be cleaved but not of the ability to be translocated.

Purification of the signal activity was followed by assaying fractions for their inhibitory activity on nascent pre-prolactin translocation (data not shown) and by electrophoresis of active fractions in polyacrylamide gels containing urea and SDS (Fig. 2).

We succeeded in isolating a tryptic fragment which was pure by the criterion of its electrophoretic mobility as a single band (Fig. 2, lane 4) and which contained signal activity (Fig. 3); it was therefore designated OV_s. The presence of OV_s in the *in vitro* translocation system reduced the ratio of prolactin to pre-prolactin (compare lane 3 with lane 1 in Fig. 3). The increased amounts of pre-prolactin that were synthesised in the presence of OV_s were clearly extra-vesicular as they were digested by post-translational proteolysis (Fig. 3, lane 4). This result ruled out the possibility that OV_s acted as a competitive inhibitor not of nascent pre-prolactin translocation but of signal peptidase (in which case one would have detected pre-prolactin molecules within microsomal vesicles, protected from post-translational proteolysis).

OV_s was then subjected to amino acid analysis. When compared (Table 1) with the amino acid composition of ovalbumin, OV_s differed by an absence of Cys, Tyr and His residues, a relative abundance of Leu, Thr, Glu/Gln, Met, Ser and Ile residues, and a paucity of Ala residues. Furthermore, OV_s contained one Lys and one Arg residue, suggesting the presence of one trypsin site that had not been cleaved.

Based on its distinct amino acid composition, OV_s could be readily and unambiguously identified within the known sequence⁹ of the ovalbumin molecule (Table 1). Thus, OV_s represents a limited tryptic fragment which contains 48 amino acid residues, beginning with Ile 229 and ending with Arg 276, and contains an uncleaved tryptic site represented by Lys 263. The relative resistance of this site to cleavage may be due to the presence of a glutamyl residue at position 262.

The localisation of signal activity in an internal rather than the amino-terminal region of mature ovalbumin was unexpected. The purification of OV_s from other tryptic fragments of ovalbumin was based on the premise that its ability to reduce the ratio of prolactin to pre-prolactin synthesised in an *in vitro*

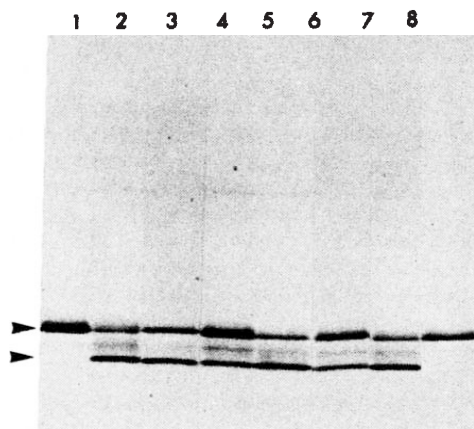


Fig. 5 RER-specific, competitive inhibition of nascent pre-prolactin translocation by OV_1 . Bovine pituitary RNA ($0.2 A_{260}$ units ml^{-1}) was translated in the nuclease-treated reticulocyte lysate in the absence or presence of various membrane fractions and OV_1 . Membrane fractions were derived either from isolated rough or smooth microsomes (both from dog pancreas)²⁶ after removal of ribosomes and nuclease treatment⁷ and are referred to in the text as RER or SER, respectively. Numbers refer to concentration in A_{260} units ml^{-1} (RER) or A_{280} units ml^{-1} (SER or OV_1). Lane 1, neither membranes nor OV_1 added. Lane 2, 1.0 RER, no OV_1 . Lane 3, 1.0 RER, 0.5 OV_1 . Lane 4, 1.0 RER, 1.0 OV_1 . Lane 5, 2.0 RER, 1.0 OV_1 . Lane 6, 1.0 RER, 1.0 SER, 1.0 OV_1 . Lane 7, 1.0 RER, 1.0 SER, no OV_1 . Lane 8, 1.0 SER, no RER, no OV_1 . Upper and lower arrowheads are as in Fig. 1.

Mode and site of action of OV_1

The structural homology of OV_1 with the signals of lysozyme and ovomucoid strongly suggested the existence of a common target represented by a signal 'receptor' domain in one of the hypothetical subunits that was proposed to participate in the assembly of the functional translocator^{1,14}.

The following experiments were carried out to establish OV_1 as a valid probe for this receptor. The approach taken was to vary the concentration of some of the components of the *in vitro* translocation system while keeping that of others constant, and to measure the effect on nascent pre-prolactin translocation⁵. Some representative data of these experiments are shown in Fig. 5, and a systematic and quantitative analysis is summarised in Fig. 6.

First, we established that OV_1 is indeed a competitive inhibitor. Increasing concentrations of OV_1 (Fig. 5, lanes 3 and 4, Fig. 6a) resulted in an increasing inhibition of nascent pre-prolactin translocation, and increased concentrations of nascent pre-prolactin at a constant concentration of OV_1 resulted in increased synthesis of prolactin (Fig. 6c). The competitive inhibition of OV_1 could be offset (Fig. 5, lane 3 versus lane 5 and Fig. 6b) by increasing the concentration of RER (microsomal vesicles derived from the rough endoplasmic reticulum).

To demonstrate the existence of a specific target for OV_1 , we took advantage of our finding here that SER (smooth microsomal vesicles which probably include Golgi membranes) is unable to translocate nascent pre-prolactin (Fig. 5, lane 8) or to compete with RER for translocation (Fig. 6a); this strongly suggests that SER does not possess a signal receptor. SER was therefore chosen as a control to rule out the possibility that OV_1 affected translocation not by its interaction with a specific signal receptor but rather, for example, by nonspecific interaction with membrane lipids. If this were the case one would expect that increasing concentrations of SER would titrate OV_1 , thereby offsetting the competitive inhibition of OV_1 on nascent pre-prolactin translocation. The data shown in Fig. 6b demonstrate that this was not so.

Timing of ovalbumin translocation

One implication of our results is that translocation of ovalbumin across membranes would be initiated relatively late in the course

of synthesis of the ovalbumin molecule. The precisely timed addition of membranes to a cell-free translation system synchronised with respect to chain elongation by the addition of an inhibitor of initiation^{15,16}, should provide information on the timing of ovalbumin translocation. In such a system an amino-terminal signal would be expected to require an early addition of microsomal membranes whereas an internal signal sequence would allow a greater length of chain to be synthesised before the growing chain would lose the capacity for functional interaction with microsomal membranes resulting in chain translocation. Our results (Fig. 7) comparing the timing of translocation of prolactin and ovalbumin are consistent with these expectations. The half-time of membrane addition (defined as the time at which translocation is reduced by 50%) was 2.0 min for prolactin and 5.5 min for ovalbumin. The half-time of chain completion (defined as the time at which 50% of the chains are completed in the synchronised system in the absence of membranes) was 5.0 min for prolactin and 8.5 min for ovalbumin (data not shown). Thus, assuming, a linear rate of synthesis, about 45 amino acid residues are incorporated per chain per min. As pre-prolactin and ovalbumin consist of 228 and 385 residues, respectively, it follows that the critical chain length after which translocation is abolished is about 90 residues for prolactin and about 250 residues for ovalbumin.

Comments

These results demonstrate that mature ovalbumin contains a latent signal sequence that can be uncovered after denaturation and can be isolated from the molecule as a tryptic fragment (OV_1) comprising amino acid residues 229–276. A striking sequence homology to the amino-terminal cleaved signals of two other hen oviduct secretory proteins (lysozyme and ovomucoid) is evident in part of OV_1 , comprising residues 234–253. It is likely, therefore, that the signal activity resides in this portion of the ovalbumin molecule. Our demonstration here that an internal signal can compete with the amino-terminal cleaved signals strongly suggests that the two have a common target, presumably a receptor domain of a protein translocator.

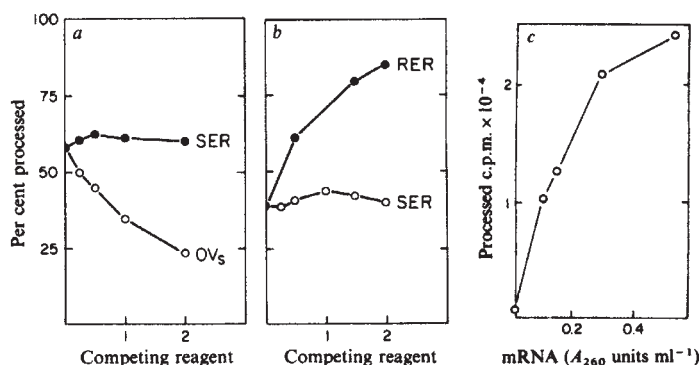


Fig. 6 Quantitative analysis of the effects of OV_1 and SER on nascent pre-prolactin translocation. *a*, A constant amount of pituitary mRNA ($0.2 A_{260}$ units ml^{-1}) was translated in the presence of a constant amount of RER ($0.8 A_{260}$ units ml^{-1}) and increasing concentrations of either OV_1 or SER. Unit concentration of competing reagent is $0.5 A_{280}$ units ml^{-1} for OV_1 and $1.0 A_{280}$ units ml^{-1} for SER. Radioactivity in gel slices was determined as previously described¹⁴. The extent of translocation is expressed as percentage of radioactivity in the prolactin band over that in the pre-prolactin and prolactin bands. *b*, A constant amount of pituitary mRNA ($0.2 A_{260}$ units ml^{-1}) was translated in the presence of a constant amount of OV_1 ($0.5 A_{280}$ units ml^{-1}) and a minimum amount of RER ($0.8 A_{260}$ units ml^{-1}). Increasing concentrations of either RER or SER were added as competing reagents in a unit concentration of $1.0 A_{280}$ units ml^{-1} for SER or $1.0 A_{260}$ units ml^{-1} for RER. *c*, Increasing concentrations of pituitary mRNA were translated in the presence of a constant amount of OV_1 ($1.0 A_{280}$ units ml^{-1}) and of RER ($1.0 A_{260}$ units ml^{-1}). The radioactivity in prolactin is shown as absolute c.p.m.

The existence of an internal signal sequence for the initiation of chain translocation has been previously postulated to explain the insertion into the RER of a viral integral membrane protein¹⁷. In this case, however, it was proposed¹⁷ that the internal signal might be cleaved by signal peptidase, ultimately yielding two integral membrane proteins. The presence in ovalbumin of an uncleaved internal signal poses the intriguing problem of how nascent ovalbumin is actually threaded through the membrane. One possibility is translocation in the form of a loop, with the signal at its leading edge, in such a way that both the left (amino-terminal) and the right (carboxyl-terminal) wings of the molecule are translocated simultaneously, as the right wing of the molecule is being completed. Other conceivable mechanisms are cotranslational translocation primarily of the right wing followed by post-translational translocation of all or the remainder of the left wing of the molecule.

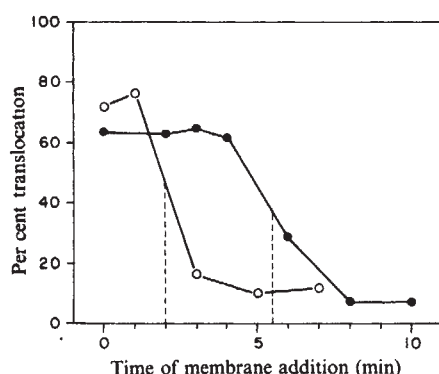


Fig. 7 Timing of translocation of prolactin and ovalbumin in a synchronised cell-free translation system. Protein synthesis was initiated in the reticulocyte lysate system. After 1 min of incubation, initiation of new chains was inhibited by the addition of 1.0 mM pactamycin¹⁵. At various times indicated on the ordinate, microsomal membranes ($5.0 A_{260}$ units ml^{-1}) were added to aliquots of the translation mixture and protein synthesis allowed to proceed to completion. ³⁵S-Methionine-labelled translation products were analysed by SDS-polyacrylamide slab gel electrophoresis and radioactivity in individual bands was determined. Per cent translocation was expressed as either (○) per cent processed prolactin (PL/PL + prePL) or (●) per cent glycosylated ovalbumin ($OV_1/OV_1 + OV_0$), as previously described⁵.

Extrapolation of such considerations to the problem of how the polypeptide chains of integral membrane proteins (IMPs) achieve their often complex orientation with respect to the lipid bilayer offers interesting theoretical solutions. To define the relationship of the polypeptide chain with the lipid bilayer, IMPs can be classified¹ as monotopic (containing hydrophilic domain(s) exposed on only one side of the lipid bilayer) or bi- and polytopic (containing two or multiple hydrophilic domains exposed on both sides of the lipid bilayer). Two examples of bitopic IMPs have been shown^{2,18} to be inserted into the

membrane through an amino-terminal cleaved signal sequence that is functionally equivalent to that of nascent secretory proteins. To abrogate complete translocation of the nascent chain, the existence of a 'stop transfer' sequence has been postulated^{2,19}. The combination of a cleaved amino-terminal signal and a stop transfer sequence further down the polypeptide chain would result in a bitopic orientation with the amino-terminal portion of the chain translocated and the carboxy-terminal portion untranslocated which, in fact, has been shown in these two cases^{2,18}). Bi- or polytopic orientations could be achieved by an internal uncleaved signal sequence combined with a stop sequence. A stop on the right wing (see above) could result in a polytopic orientation whereby only an internal portion of the molecule is translocated, leaving substantial portions of both wings (containing amino and carboxy terminus) untranslocated. A stop on the left wing could result in a completely translocated right wing and an untranslocated left wing, leaving the amino-terminal portion of the molecule untranslocated. More complex polytopic orientations could be achieved by alternating multiple signal and stop sequences¹⁷.

It is interesting that the last of the seven intervening sequences in the ovalbumin gene²⁰ is located between residues 254 and 255, that is, near the eliminated signal peptidase site. An intervening sequence near the signal peptidase site is also present in the gene for the light chain of IgG²¹. Thus, signal sequences and also stop transfer sequences could have evolved as a result of gene amplification and recombination¹.

The isolation of an internal signal is clearly of practical importance. OV_1 has already been used here as a probe to demonstrate that its target is located in the rough and not the smooth endoplasmic reticulum. Experiments are in progress to study in detail the interaction of OV_1 with the RER, in the hope that it can be used as an affinity ligand for the purification of its target.

Finally, the demonstration here of an internal uncleaved signal sequence with the same target in the RER as the amino-terminal cleaved signals, is relevant to currently used nomenclature. Retrospectively, it turns out that the term 'signal' was a more accurate choice³ than designations such as leader sequence, pre-sequence or extra piece, all of which have structural implications such as amino-terminal location and/or cleavage. Thus, we propose that the term signal sequence be reserved to define a segment of the polypeptide chain, cleaved or uncleaved, which triggers protein translocation across a cellular membrane by virtue of its interaction with a specific translocator. Although it seems that a single translocator exists for the co-translational transfer of secretory and membrane proteins across the membrane of the RER, recent evidence indicates²²⁻²⁴ that eukaryotic cells also contain several other specific translocators, localised to other distinct cellular membranes, for the recognition of structurally unique signals for the post-translational import of proteins from the cytosol into organelles.

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A structurally abnormal insulin causing human diabetes

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Insulin isolated from the pancreas of a diabetic patient with fasting hyperinsulinaemia showed decreased activity in binding to cell membrane insulin receptors and in stimulating cellular 2-deoxyglucose transport and glucose oxidation. Chemical studies suggest that the isolated hormone is a mixture of normal insulin and an abnormal variant which contains a leucine for phenylalanine substitution at position 24 or 25 of the insulin B-chain.

INSULIN RESISTANCE, a clinical syndrome identified by an individual's failure to respond normally to endogenously secreted and exogenously administered insulin, can lead to severe diabetes even when plasma levels of the hormone are elevated. In many patients, this 'resistance' is due to a target cell abnormality or to the presence of circulating insulin antagonists¹. We and others have described conditions in which cellular defects involve decreased numbers of membrane-associated insulin receptors in peripheral tissues² or decreased cellular responsiveness at a step subsequent to hormone-receptor interaction³. Circulating antagonists which lead to insulin resistance include anti-insulin antibodies⁴, anti-insulin-receptor antibodies^{5,6} and counter-regulatory hormones at high levels^{1,7}. Patients exhibiting elevated plasma ratios of proinsulin to insulin⁸ may also seem to be insulin resistant, but in these individuals the peripheral concentration of the 5,800-molecular weight hormone is lower than indicated by direct radio-immunoassay, and the patients actually respond normally to their plasma concentrations of free insulin.

We recently identified a patient (R.C.) with diabetes and hyperinsulinaemia whose disease did not seem to fit any of the above descriptions. Our findings, which will be described in detail elsewhere, showed that (1) the patient exhibited a fasting plasma glucose level of >200 mg dl⁻¹ (normal, 80–120 mg dl⁻¹) and a fasting, plasma insulin level of 70–120 μ units ml⁻¹ (normal, 5–24 μ units ml⁻¹); (2) the ratio of proinsulin to insulin in the patient's plasma was normal; (3) the patient's plasma did not contain anti-insulin or anti-insulin-receptor antibodies, nor elevated concentrations of insulin antagonists such as growth hormone, glucagon or cortisol; (4) the patient responded to exogenously administered insulin in a normal manner; (5) monocytes isolated from the patient's blood contained normal numbers of insulin receptors; (6) insulin isolated from the patient's plasma by immunoaffinity chromatography had a decreased ability to bind to both cultured IM-9 lymphocytes and rat adipocytes; (7) the plasma insulin had lower than normal activity in stimulating glucose uptake and oxidation in rat adipocytes. Although the possibility of a detrimental modification of the patient's insulin by a plasma factor could not be excluded, we considered it most likely that the patient

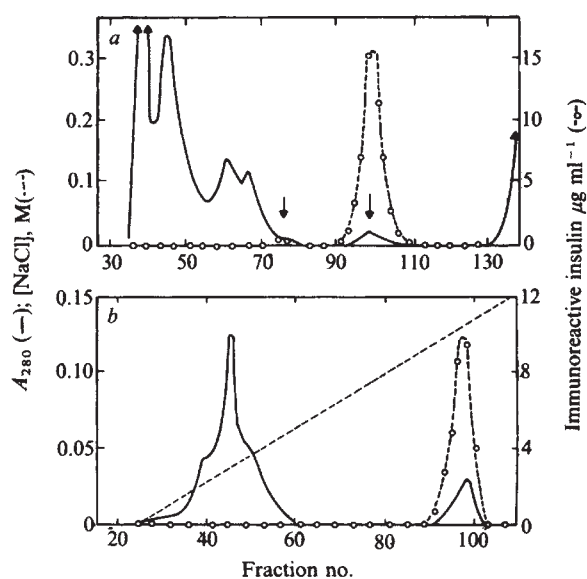


Fig. 1 Purification of pancreatic insulin from patient R.C. *a*, Insulin, partially purified by the method of Davoren⁹, was dissolved in 5 ml of 3 M acetic acid and gel filtered on a column (2.5 × 90 cm) of BioGel P-30 (100–200 mesh, Bio-Rad) which had been equilibrated with the same solvent. The flow rate was 20 ml h⁻¹ and 3-ml fractions were collected. Absorbance at 280 nm and insulin immunoreactivity were measured in the eluted fractions. The left and right vertical arrows show the elution positions of proinsulin and insulin, respectively. *b*, Insulin-containing fractions from the profile of *a* were pooled and the 30 ml of solution were applied to a column (0.9 × 20 cm) of SP-Sephadex C-25 (sodium form, Pharmacia) which had been equilibrated with 3 M acetic acid. The column was washed with 50 ml of the solvent and a linear gradient of NaCl dissolved in 3 M acetic acid (0–0.25 M, 200 ml of each) was applied to the column at a flow rate of 30 ml h⁻¹. Fractions containing 3 ml were collected and absorbance at 280 nm and insulin immunoreactivity were measured.

secreted an abnormal insulin, the structure of which had been modified by genetic mutation. During exploratory surgery for the examination of an abdominal mass with pancreatic involvement, a portion of the patient's pancreas was removed for histological study. The portion remaining after completion of the pathological examination was made available to us for biochemical analysis. This report describes the isolation and characterisation of the patient's pancreatic insulin.

Isolation of insulin

A 3.1-g specimen of pancreas from patient R.C. was homogenised in acidified ethanol and the insulin partially purified by

Table 1 Amino acid composition of isolated insulin and insulin fragments

Amino acid	mol amino acid per mol peptide					
	Insulin		Desoctapeptide insulin		Heptapeptide B ₂₃₋₂₉	
	Found	Theory	Found	Theory	Found	Theory
Aspartic acid	3.2	3	3.2	3		
Threonine	2.8	3	1.2	1	1.0	1
Serine	2.5	3	2.9	3		
Glutamic acid	7.2	7	7.0	7		
Proline	1.1	1			1.0	1
Glycine	4.3	4	3.7	3	0.9	1
Alanine	1.0	1	1.3	1		
Cysteine	ND	6	ND	6		
Valine	3.9	4	3.6	4		
Isoleucine	1.9	2	1.6	2		
Leucine	6.8	6	6.0	6	0.6	
Tyrosine	4.0	4	3.0	3	1.0	1
Phenylalanine	2.3	3	0.9	1	1.4	2
Histidine	2.5	2	1.8	2		
Lysine	1.4	1			1.1	1
Arginine	1.1	1	1.0	1		

Peptides were hydrolysed under vacuum at 110 °C for 20 h in 6 M HCl containing a small amount of phenol. Analyses were carried out on a Durrum amino acid analyser. Relative amino acid concentrations were converted to nearest integer values to obtain the compositions reported in columns labelled 'Found'. Columns labelled 'Theory' show the known compositions of the normal human peptides. Cysteine content was not determined (ND).

the method of Davoren⁹. Material obtained by ether-alcohol precipitation was dissolved in 3M acetic acid and the resulting solution was gel-filtered on BioGel P-30 to yield the profile shown in Fig. 1a. Our finding that approximately 95% of the insulin-like immunoreactivity appeared as a single component having the size of native insulin (fractions 91–109) indicates a normal ratio of pancreatic proinsulin to insulin. To avoid additional methods of purification either requiring large amounts of material, such as crystallisation, or resulting in low yields, such as paper electrophoresis, we developed the specialised use of cation exchange chromatography for the final purification of the hormone. Fractions 94–105 from the profile of Fig. 1a were pooled and applied without concentration to a column of SP-Sephadex which had been equilibrated with 3M acetic acid. Application of a gradient of NaCl to the column resulted in the absorbance and immunoreactivity profiles shown in Fig. 1b. The peak of immunoreactive insulin was well separated from earlier eluting material which included the proinsulin C-peptide as well as other contaminating peptides. The yield of insulin, after pooling and desalting fractions 92–101, was approximately 50 nmol as measured either by absorbance at 275 nm or by colorimetric methods¹⁰. This value, which is equivalent to a yield of 98 mg per kg of tissue, represents the expected content of the hormone in pancreatic tissue¹¹.

To standardise the preparation further, identical amounts of the isolated insulin and monocomponent porcine insulin, as determined by the Lowry protein assay¹⁰, were diluted in parallel and examined by radioimmunoassay¹². As shown in Fig. 2, the immunometric potencies of the patient's insulin and porcine insulin were indistinguishable over a wide range of concentration. This result indicates the high degree of purity of the isolated insulin and validates the use of our radioimmunoassay in its measurement. Experiments not illustrated here also showed that the mobility of the patient insulin during polyacrylamide gel electrophoresis at pH 8.7 (ref. 13) or pH 4.5 (ref. 14) was indistinguishable from that of normal human insulin. As both gel systems are capable of resolving peptides having single charge differences, we could disregard the possibility that the preparation was contaminated by desamido- or arginyl-insulin, or that charged residues within the patient

insulin had been modified. Glucagon was not detectable by sensitive radioimmunoassay in the final preparation.

Biological activity

The biological effectiveness of the pancreatic insulin from patient R.C. was compared with that of monocomponent porcine insulin on the basis of immunometric equivalency. This comparison is validated by the results of Fig. 2 and by our previous studies which have shown that porcine insulin and normal human insulin are equipotent when tested in any of the receptor-binding or biological assay systems used^{3,25}. As illustrated in the experiments of Fig. 3, the patient insulin did not compete as well as normal insulin did for the binding of ¹²⁵I-labelled porcine insulin to cultured, human IM-9 lymphocytes or to isolated rat adipocytes. The shapes of the respective inhibition curves, however, were similar to those obtained using normal insulin. The decreased effectiveness of the patient insulin in these systems was confirmed on many occasions for the hormone isolated from both pancreas and plasma. Average potencies were 46 ± 8% of normal (average ± s.d.) for binding to IM-9 lymphocytes (*n* = 9) and 51 ± 8% of normal for binding to rat adipocytes (*n* = 7). Further experiments were designed to assess the biological activity of the insulin from patient R.C. Figure 4 shows that, whether activity was measured by stimulation of 2-deoxyglucose transport or by stimulation of ¹⁴C-1-glucose oxidation, the patient insulin showed greatly decreased biological activity. Its potency, assessed by estimating that concentration causing half-maximal stimulation, was only 11 ± 5% (*n* = 8) and 12 ± 5% (*n* = 14) of normal in the two respective systems. Nevertheless, at high concentrations (10–50 ng ml⁻¹) the patient insulin was fully effective at stimulating these cellular processes. This decreased biological activity, as well as the decreased ability of the insulin to bind to membrane receptors, suggested that the pancreatic insulin from patient R.C. was structurally different from normal human insulin.

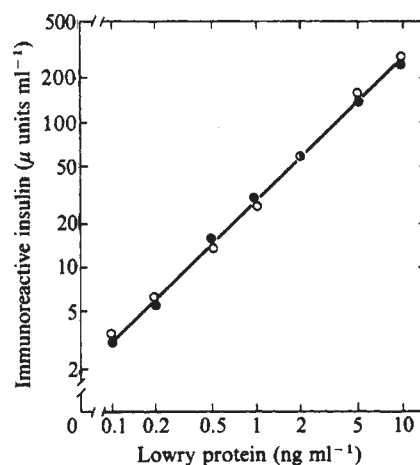


Fig. 2 Immunoreactive potency of the patient insulin. The protein concentration of the solution of patient insulin was determined by the method of Lowry¹⁰ using a standard curve derived from porcine insulin. Amounts of the patient insulin and monocomponent porcine insulin corresponding to 1 μg of protein were diluted in parallel to give the concentrations shown on the abscissa. Aliquots (1 ml) of each solution were then subjected to the radioimmunoassay in standard conditions¹². Averages of duplicate immunoreactivity determinations for the patient insulin (○) and monocomponent porcine insulin (●) are shown.

Chemical characterisation

Treatment of the purified patient insulin with 1-dimethylaminonaphthalene-5-sulphonyl chloride followed by subsequent analysis¹⁹ led to identification of glycine and

phenylalanine as the NH_2 -terminal residues of the peptide. Amino acid analysis was carried out on the isolated peptide to probe a possible structural alteration in the patient insulin. As shown in the first column of Table 1, the compositional values obtained are very close to those expected for human insulin, with the exceptions that the content of leucine was high and that of phenylalanine low. The sources of the slightly elevated values for histidine and lysine are not known, but the presence of these residues in the patient insulin could be excluded because the peptide showed the expected electrophoretic mobilities during polyacrylamide gel electrophoresis. As NH_2 -terminal analysis showed both phenylalanine and glycine, we could assign one residue of phenylalanine to position 1 of the insulin B-chain. In the simplest case, any decrease in phenylalanine content must then result from an amino acid substitution at position B_{24} or B_{25} . Fortunately, these residues occur in the COOH-terminal octapeptide of the insulin B-chain, a region which is easily excised by proteolytic digestion.

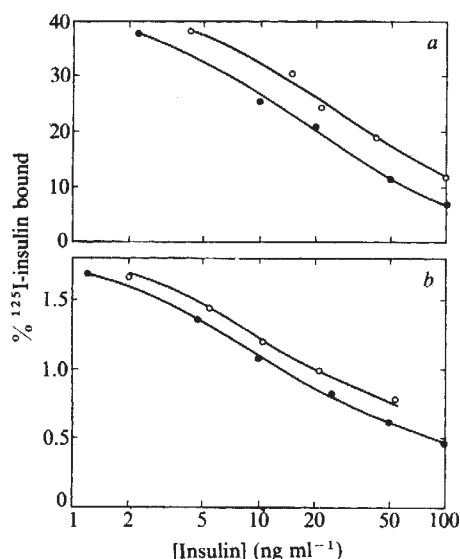


Fig. 3 Competition for the binding of ^{125}I -labelled porcine insulin to plasma membrane receptors by the patient insulin. *a*, Binding of insulin to cultured, human IM-9 lymphocytes; *b*, binding of insulin to isolated, rat adipocytes. Methods for the preparation of cells and for the binding studies using lymphocytes¹⁶ and adipocytes^{15,17,18} have been described. In each case, the potency of the patient insulin ($\circ$) was compared with that of monocomponent porcine insulin ($\bullet$) at the concentrations shown.

The patient insulin ($100\text{ }\mu\text{g}$) was incubated at 30°C for 3 h with $10\text{ }\mu\text{g}$ of tosylphenylalanine chloromethylketone-treated trypsin in 0.2 ml of 0.1 M *N*-ethylmorpholine brought to $\text{pH } 8$ with acetic acid. The resulting desoctapeptide insulin (lacking residues B_{23-30}), heptapeptide (containing residues B_{23-29}), and threonine (residue B_{30}) were separated by paper electrophoresis in the above *N*-ethylmorpholine buffer; peptides were subsequently eluted in 50% acetic acid. Amino acid analysis of the desoctapeptide insulin demonstrated, as expected, the presence of a single residue of phenylalanine (Table 1, column 3). The amino acid composition of the heptapeptide B_{23-29} (Table 1, column 5) shows values consistent with theory for glycine, tyrosine, threonine, proline and lysine. The peptide was deficient, however, by the equivalent of 0.6 residues of phenylalanine and contained the equivalent of 0.6 residues of leucine, an amino acid not expected from theory, nor found when we applied identical procedures to the preparation and analysis of the heptapeptide from porcine insulin. These results localise the leucine for phenylalanine substitution, which was suggested by the amino acid composition of the intact insulin, to the COOH-

terminal octapeptide of the B-chain. Our finding of less than a full residue of leucine in the heptapeptide, however, suggests a limited degree of microheterogeneity in the isolated hormone. As we wished to conserve the remaining small amount of insulin for use as a structural probe of the insulin receptor, and as the amino acid substitution could only have occurred at one or the other of the two adjacent phenylalanine residues of the heptapeptide (B_{24} or B_{25}), we did not attempt to place the substitution in sequence.

Discussion

Our results show that the pancreatic insulin isolated from a patient who was resistant to endogenous insulin, but who responded normally to exogenous insulin, had a substantially lower than normal biological activity. These findings extend our observation that insulin isolated from the patient's plasma was of low potency and identify the defect in the hormone as occurring before its secretion. The leucine for phenylalanine substitution, which we localised in the COOH-terminal region of the B-chain of the patient insulin, represents a single base change which might have occurred by either of two point mutations, regardless of which of the phenylalanine codons were present in the gene sequence. This statistically favourable mutation undoubtedly results in the decreased biological effectiveness associated with the isolated insulin. Our finding of lower than integral values for the content of phenylalanine and leucine in the patient insulin, and a preliminary observation that the ^{125}I -labelled patient insulin is divisible into two components based on ability to bind to membrane receptors, suggest that the isolated hormone consists of normal human insulin and the mutant insulin in the approximate ratio $40:60$. We consider that this mixture probably represents the products of the nearly co-dominant expression of two allelic genes in the heterozygous state; it is thus likely that identical maternal and paternal insulin genes are expressed simultaneously in normal individuals. In the absence of a more complete genetic analysis, however, we cannot exclude the possibility that the microheterogeneity, in

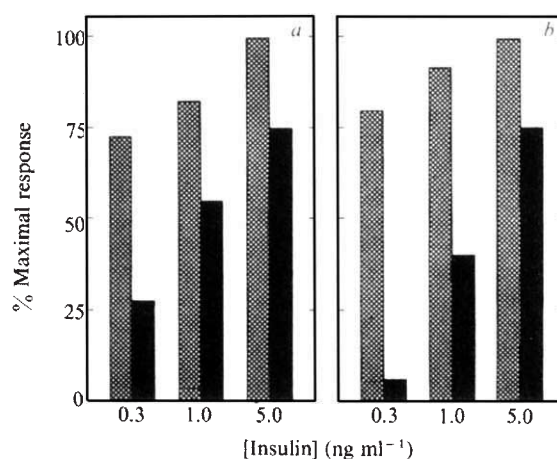


Fig. 4 Biological activity of the patient insulin. *a*, Stimulation of ^{14}C -2-deoxyglucose transport into isolated, rat adipocytes; *b*, stimulation of ^{14}C -glucose oxidation by isolated, rat adipocytes. Methods for the transport and glucose oxidation experiments have been described^{12,15}. In each case, the potency of the patient insulin (solid bars) is compared with that of monocomponent porcine insulin (hatched bars) at the selected concentrations shown. Basal and maximally stimulated values for 2-deoxyglucose transport were 0.13 and $0.51\text{ nmol per } 2 \times 10^5\text{ cells per } 3\text{ min}$, respectively. Basal and maximally stimulated values for glucose oxidation were 3 and $61\text{ nmol per } 2 \times 10^5\text{ cells per h}$, respectively.

this selected case, results from the expression of two non-allelic genes, as is found for the two insulins of the rat^{20,21}.

Although we have not placed the proposed amino acid substitution in sequence, its localisation to position 24 or 25 of the insulin B-chain, as illustrated diagrammatically in Fig. 5, is particularly interesting. First, the B-chain sequence, Gly-Phe-Phe-Tyr, residues 23–26, has remained invariant during animal evolution, even when considering the hormones derived from the guinea pig²¹ or the primitive hagfish²². No other sequence lacking cysteine residues and having comparable length seems to be as stable from an evolutionary point of view. Second, using various criteria, residues 23–26 of the COOH-terminal region of the B-chain have been placed in the active site of the hormone. Particularly important are the findings that the biological activity of modified insulins decreases progressively as from five to eight residues are removed from the COOH terminus of the B-chain²³ and that synthetic peptides containing residues B_{22–26} have low amounts of insulin-like activity²⁴. The effects of replacing phenylalanine by leucine within this sequence seem to be quite specific, as this change has little effect on the general hydrophobic character of the region. Third, the COOH-terminal B-chain sequence containing residues 24–26 has been assigned to the negatively cooperative site for the interaction of the hormone with its receptor²⁵. Fourth, this region of the B-chain has a crucial role in dimer formation within the zinc-insulin hexamer²⁶. Note, however, that electron micrographs of islet tissue from patient R.C. revealed well defined crystalline inclusions typical of insulin-containing secretion granules. Other aspects of pancreatic and insular morphology were also unremarkable.

The low biological activity of the patient insulin (11–12% of normal), in comparison with its receptor binding activity (46–51% of normal), represents an unexpected result. Similar findings have been reported for the action and binding of hagfish insulin in mammalian systems (ref. 27 and S. Emdin, personal communication), but these findings have been questioned²⁸. Notwithstanding this discrepancy, our results might be explained by a multi-step mechanism of insulin action allowing a degree of dissociation between receptor binding and hormone action, or by an antagonism of the action of the normal insulin by the abnormal variant in the preparation used. Because the apparent binding potency of the patient insulin corresponds well with that expected from the amount of normal insulin present in the mixture, the latter possibility seems the more likely. The very low biological activity of the patient insulin further suggests that this proposed antagonism occurs in a multiplicative way; that is, one molecule of the variant apparently inhibits the action of several molecules of normal insulin. This model would have important implications in the study of the mechanism of insulin action.

The relationship between the insulin variant and our patient's diabetes is important. Considerations of spare insulin receptors might have predicted a physiological compensation by the secretion of additional amounts of the hormone. The antagonis-

tic behaviour of the variant in the secreted hormonal mixture suggests, however, that such compensation might not have been possible *in vivo*. It is likely that the increased ratio of active insulin to the variant produced by the insulin tolerance test was crucial in correcting the patient's glucose intolerance in our clinical studies. Our description of an abnormal insulin suggests the usefulness of the cataloguing of diabetes in hyperinsulinaemic states in which the disease can be ascribed to (1) a mutation in the structural gene for (prepro)insulin, (2)

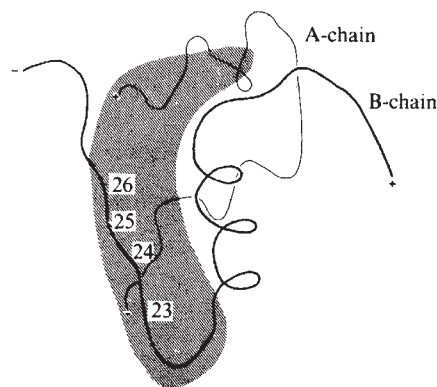


Fig. 5 Diagrammatic representation of the structure of insulin. The simplified drawing is derived from the crystallographic and other studies of Blundell *et al.*²⁹. The biologically important, active site is indicated by hatching, and the positions of the invariant residues B_{23–26} (Gly-Phe-Phe-Tyr) are shown.

incomplete conversion of proinsulin to insulin, (3) circulating antagonists to insulin, (4) defects in the insulin receptor site, and (5) defects in target cell responsiveness at a site distal to hormone-receptor binding. Identification of the biochemical abnormality in any individual thus requires a comprehensive evaluation of these various possibilities. We are now examining plasma insulin from other patients whose insulin resistance mimics that of patient R.C. Use of these insulins as naturally occurring analogues is likely to provide further insight into the structural requirements for the binding of insulin to its receptor and for its activation of cellular processes.

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letters

The high-latitude EUV source HD192273 as a low-mass binary

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The B star HD192273 was tentatively identified¹ as an extreme-ultraviolet (EUV) source. Photometry² and photographic spectra of this star show that the optical and EUV fluxes fit a model with $T_e = 27,000$ K and $\log g = 4.8$, values reasonably close to those of a normal B2 V star. However, if HD 192273 is such a normal main-sequence star, its distance D is ~ 4 kpc, difficult to reconcile with the low line-of-sight column density ($< 10^{18} \text{ cm}^{-2}$ over the 4-kpc path), the low radial velocity ($\sim 24 \text{ km s}^{-1}$), and the high distance (~ 2 kpc) out of the galactic plane. Instead, we suggest that this object is an analogue of HZ22, an evolved, low-mass component of a binary³, where the spectroscopic $\log g$ is consistent with a relatively small radius ($0.1\text{--}1 R_\odot$ and a small distance D of 100–200 pc. Such a suggestion can explain both the low H I column density and the small radial velocity.

Trimble's⁴ calculations of the evolution of evolved binary components like HZ22 show that following mass exchange, stars with helium cores and thin hydrogen envelopes spend appreciable times ($10^3\text{--}10^6$ yr, with longer lifetimes at lower masses) near the He main sequence. Using Wegner's spectroscopic value of $\log g = 4.8$,

$$\log R/R_\odot = -0.7 + 0.5 \log (M/0.1M_\odot) \quad (1)$$

Trimble's lowest-mass models with $M/M_\odot = 0.26$ fall reasonably close to the radius specified by equation (1). The spectroscopic temperature of 27,000 K specifies the monochromatic Eddington flux as $2.52 \times 10^{-4} \text{ erg cm}^{-2} \text{ Hz}^{-1}$ (ref. 5). Adopting the relation between flux, magnitude, radius, and distance described in ref. 6, using equation (1) and taking $V = 8.827$, we obtain:

$$D = 80(M/0.1M_\odot)^{1/2} \text{ pc} \quad (2)$$

The precise value of D depends more on M and less on T and $\log g$. We suggest that M is a few tenths of a solar mass giving $D = 100\text{--}200$ pc.

Wegner² derived a maximum H I column density of $5 \times 10^{17} \text{ cm}^{-2}$ from fitting a $T_e = 28,630$ K model to the observations of the EUV flux. Some exploration of the sensitivity of the upper limit to assumptions regarding T_e and $\log g$ is warranted, because the dependence of the EUV flux on T_e is quite steep, (the flux at 600 Å increases by a factor of 100 between $T_e = 27,500$ K and $T_e = 32,500$ K in Mihalas' models⁷). A maximal temperature of 32,500 K, $\log g = 4$, and a non-LTE model maximises the EUV flux at the stellar surface in a way consistent with the optical observations. Such a model gives $F(600 \text{ Å})/F(5,500 \text{ Å}) = 0.27$ at the star, which can be reduced

to the observed value (at the Earth) of 0.0034 if the optical depth at 600 Å to HD192273 is 2.

Such an optical depth corresponds to a column density of 10^{18} H I atoms cm^{-2} using the cross-sections of Cruddace *et al.*⁸. This limit is insensitive to the details of the analysis. No matter what kind of an EUV emitter this source is, the optical depth to it cannot be too high without making it invisible.

Is this interstellar column density reasonable, given current conceptions and observations of the interstellar medium? Consider two models: (1) HD192273 is a normal, main-sequence B star with $M_V = -4$ at a distance of 3.6 kpc (ref. 9) and (2) the present suggestion that it is a low-mass binary at a distance of 100–200 pc. Bohlin *et al.*¹⁰ provides a survey of interstellar N(H I) based on observations of Lyα. Their Fig. 5 contains observations of stars far from the galactic plane, showing positive detections of N(H I) exceeding 10^{20} cm^{-2} for seven high-latitude stars with distances exceeding 100 pc. Were HD 192273 a normal B star, its distance of 3.6 kpc would place it far beyond all the stars observed by Bohlin *et al.* We therefore conclude that model (1) is inconsistent with the data from interstellar Lyα observations.

However, if HD192273 were much closer, the interstellar column density required is compatible with the observations of other EUV sources. The upper limit to the column density towards HD192273 is only slightly smaller than the values of the column density obtained for HZ43 [where $0.8 \times 10^{18} < N(\text{H I}) < 2.5 \times 10^{18}$ (ref. 11)], and Feige 24 [where N(H I) can vary between 10^{18} and $5 \times 10^{18} \text{ cm}^{-2}$ (H.L.S., unpublished) or is less than 3×10^{18} (ref. 12).] The distances of HZ43 and Feige 24 are 60 pc (ref. 13) and 60–140 pc (ref. 14) respectively, comparable to the distance to HD192273 if it is a low-mass binary. Bohlin *et al.*¹⁶ provide little information on such nearby objects, but the reasonably well established column densities towards HZ43, Feige 24, and the density towards HD192273 if model (2) is correct seem consistent with their observations, particularly considering that the neutral hydrogen is concentrated in a few clouds.

This interpretation of HD192273 is supported by two other pieces of information. Its spectroscopic $\log g$, although uncertain, seems slightly high for a main-sequence B star. Furthermore, its low radial velocity would present problems were it 2 kpc from the galactic plane. Greenstein and Sargent¹⁵ discuss a fairly large sample of similarly paradoxical high-latitude B stars. Their Table 4 provides an indication of the time required for stars with various radial velocities and at various heights z above the galactic plane to reach their present positions. FB180 and FB181, with z -distances of 1.6 and 1.9 kpc and $\rho'(z) = -31$ and -21 km s^{-1} respectively, are similar to HD192273 as a main-sequence B star with $z = 1.8$ kpc and $\rho' = -27 \text{ km s}^{-1}$. (Here $\rho'(z)$ is the component of the radial velocity in the z -direction, corrected for solar motion.) Both FB180 and FB181 would take 4×10^7 yr to reach their present positions, and so we infer that a similar time scale applies to HD192273 if model (1) is correct. But as a main-sequence star, HD192273 has a lifetime of only 6×10^6 yr, and thus there is no obvious way that it could come to be where it is. However, if HD192273 is a low-mass binary component 100–200 pc away (model 2), the low radial velocity presents no problems.

This suggestion that HD192273 is a low-mass binary, combined with the spectroscopically confirmed binary nature of

HZ22, prompts a speculation that many of the high-latitude O and B stars with low radial velocities and apparently large distances could be similar low-mass binaries at considerably smaller distances. This picture of HD192273 as a low-mass binary could be confirmed by a radial velocity study, although the absence of radial velocity variations would not rule out the present interpretation.

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A limit to the X-ray luminosity of nearby normal galaxies

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The origin of the extragalactic diffuse X-ray background is not well understood. It is isotropic to the extent that a galactic origin can be excluded. Indeed, even a halo of radius as large as 12 kpc could provide no more than 3% of the flux¹. The spectrum between 3 and 50 keV fits a 40-keV thermal bremsstrahlung form which is suggestive of emission from a hot intergalactic medium². This or some other truly diffuse mechanism may be responsible. If, however, discrete sources play a major part, we require either strong evolution of an already recognised X-ray emitting class, such as quasars, or as we consider here, a hitherto elusive population of low luminosity emitter.

Without invoking evolution, the largest background contributions are thought to derive from Seyfert galaxies and clusters of galaxies. Based on estimated local densities, the total background cannot be accounted for^{2,3}. Consistency with fluctuation measurements of the diffuse background is only achieved if members of the additional non-evolved class of source, which would be required to complete the background, are sufficiently numerous and hence of low luminosity. Compatibility requires a volume density $\geq 8 \times 10^{-3} h^3 \text{ Mpc}^{-3}$ (ref. 3), where h is the present Hubble constant in units of $100 \text{ km s}^{-1} \text{ Mpc}^{-1}$. The diffuse background between 2 and 10 keV has an emissivity $\sim 4.7 \times 10^{39} h \text{ erg s}^{-1} \text{ Mpc}^{-3}$, which places an upper limit of $\sim 6 \times 10^{41} / h^2 \text{ erg s}^{-1}$ on the source luminosity. (We quote luminosities between 2 and 10 keV to ease comparison with other work. However, the source and diffuse background data used here are both from the HEAO A-2 experiment and comparison will be made over the entire energy range for which we have a response, that is 2–60 keV; with highest efficiency 3–20 keV.)

Such weak extragalactic sources do not present a high detection probability for non-imaging proportional counter experiments. The requirements for source detection with the A-2 detectors on the HEAO 1 spacecraft are discussed in ref. 4.

There is dependence on ecliptic latitude and consequent exposure but a 5σ detection threshold is $\sim 2 \times 10^{-11} \text{ erg cm}^{-2} \text{ s}^{-1}$ (2–10 keV) requiring, for example, a $10^{40} \text{ erg s}^{-1}$ emitter to be within $\sim 2 \text{ Mpc}$. To date the only catalogued galaxies $< 10^{42} \text{ erg s}^{-1}$ are SMC, LMC, M31, Cen A and M82. Of these, the latter two are radio galaxies but are, probably, atypical of their class in general. The others are classed as normal galaxies and their X-ray luminosity is compatible with that of our Galaxy, estimated, from the summed emissivity of known sources, to be $\sim 2 \times 10^{39} \text{ erg s}^{-1}$. Taking M31 and the Galaxy as standards, normal galaxies are estimated to contribute only a few per cent of the diffuse background^{3,5,6}. However, as the most numerous extragalactic object they merit further examination in the search for a class of low luminosity X-ray source.

We report here results of a test of the hypothesis that normal galaxies are on average more luminous than our Galaxy and M31 and therefore form a candidate class of numerous low luminosity emitters which may fulfill requirements for contributing substantially to the diffuse background. The imaging experiments on the Einstein Observatory will measure at energies below $\sim 4 \text{ keV}$ where low-energy absorption and soft sources will limit comparison with the 2–60 keV background. As noted above, the comparison between source and background fluxes are made over the HEAO A-2 experiment energy range. But, where 2–10-keV source luminosities are given, a constant multiplicative factor suitable for a 9-keV thermal bremsstrahlung spectrum has been used.

Data from the A-2 detectors on the HEAO 1 spacecraft⁷ were investigated for emission from positions coincident with normal galaxies selected from the *Second Reference Catalogue of Bright Galaxies*⁸. Selection required a velocity $< 1,100 \text{ km s}^{-1}$ (that is $D < 20 \text{ Mpc}$ for $H_0 = 55 \text{ km s}^{-1} \text{ Mpc}^{-1}$). Positions contaminated by catalogued X-ray sources were excluded. Since only data from the $1.5^\circ \times 3^\circ$ field of view detectors were used, a source free region 3° each side of the galaxy along the scan direction and 5° in the perpendicular direction was demanded. Where galaxies were clustered the closest member was selected. Seventy-six galaxies satisfied requirements. A 25° field of scan angle about the galaxy position, accumulated over ~ 4 days about the one of maximum exposure, was fitted with a fixed source at the galaxy position and as many other sources as necessary to fit the remainder of the field. Negative values for the source count rate were allowed. After correcting for effective exposure for each fixed source a count rate distribution was found. This was consistent, to within 1σ errors, with zero. This result implies that

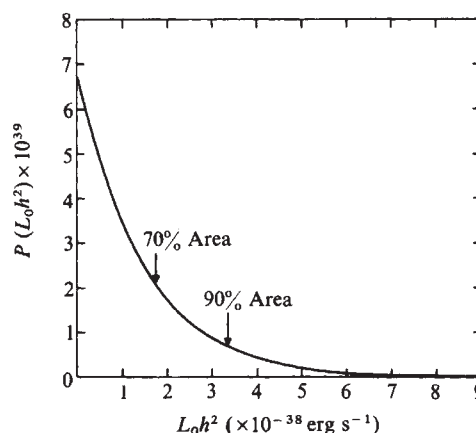


Fig. 1 The $L_0 h^2$ probability function resulting from the application of a luminosity function $F(L, L_0) = (1/L_0 h^2) \exp(-L/L_0 h^2)$ to the sample of 76 normal galaxies. The result fits the indicated exponential function. The values of $L_0 h^2$ bounding 70% and 90% of the area are shown, that is the upper limits to $L_0 h^2$ at these respective confidence levels. $P(L_0 h^2) \propto \exp(-L_0 h^2 / 1.46 \times 10^{38})$.

we can only determine upper limits to the average luminosity for various model luminosity distributions.

The simplest model which can be examined is one in which all galaxies are assumed to have a standard luminosity, Lh^2 . Then the probability of observing the i th member of the 76 galaxies with its measured luminosity, $L_i h^2$, and associated standard deviation, σ_i , is $P_{si}(Lh^2) = (\sigma_i(2\pi)^{1/2})^{-1} \exp(-z_i^2/2)$, where $z_i = (L_i - L)h^2/\sigma_i$. Hence the likelihood function for the measured luminosities of the complete sample is $P_s(Lh^2) \propto \exp(-\sum_i(z_i^2/2))$. The values for σ_i were calculated from the standard deviation of the count rate distribution for the selected galaxies after this value had been confirmed to be consistent with values derived using random sample positions on the sky. The function $P_s(Lh^2)$ is of a gaussian form. The values of Lh^2 bounding 70% and 90% of the area are $1.5 \times 10^{38} \text{ erg s}^{-1}$ and $2.7 \times 10^{38} \text{ erg s}^{-1}$ respectively, which are the upper limits to the standard normal galaxy luminosity at these respective confidence levels. Even for a value of H_0 of $50 \text{ km s}^{-1} \text{ Mpc}^{-1}$ the results show that the average X-ray luminosity of normal galaxies is $\leq 10^{39} \text{ erg s}^{-1}$. This is a little lower than the X-ray luminosity of our Galaxy. However, we note that the average optical luminosity of the chosen galaxies in the B system is $(2 \times 10^9/h^2)L_\odot$ and the appropriate value for our Galaxy with which this should be compared is $11 \times 10^9 L_\odot$ (the mean orientation value estimated from the galactic pole value given in ref. 9).

More realistically, the luminosity function of the galaxies can be assumed to be an exponential, $F(L, L_0) = (L_0 h^2)^{-1} \exp(-L/L_0 h^2)$. The probability of observing the i th member of the 76 galaxies with its measured luminosity is now $P_i(L_0 h^2) = \int F(L, L_0) P_{si}(Lh^2) dL$. The product over all values of i gives the likelihood function for the complete sample. As shown in Fig. 1, it is approximately exponential. The 70% and 90% upper limits to $L_0 h^2$ are now $1.8 \times 10^{38} \text{ erg s}^{-1}$ and $3.4 \times 10^{38} \text{ erg s}^{-1}$, in reasonable agreement with the simple model.

The method generates a strong distance-dependent weighting factor. Because the answer is dominated by the closest 10 galaxies, selection effects may be important. To investigate this we divide the galaxies by distance into three bands. The number of galaxies per band increases with distance so that the three calculated luminosity upper limits are roughly equal. We find that they are ~ 3 times those quoted above. Thus we conclude that, to within a factor of 3, the 76 galaxies form a homogeneous class of which the closest 10 are not particularly anomalous. This is further confirmed in that the mean count rate for these 10 is within 1σ of the mean of the total sample.

Based on these results, we can make an h -independent comparison of the emissivity of galaxies with that of the diffuse background. The density of normal galaxies is calculated to be $\approx 0.13 h^3 \text{ Mpc}^{-3}$. This uses a likely maximum value for the optical luminosity density of galaxies of $2.6 \times 10^8 h L_\odot \text{ Mpc}^{-3}$ (see ref. 10) and the average luminosity for our sample as given above. For the detector fields of view we are using (defined as R15 in ref. 4) and applying the 90% upper limit criterion for the exponential luminosity distribution model, we find an emissivity of $< 2.7 \times 10^{45} h \text{ ct s}^{-1} \text{ Mpc}^{-3}$. This is to be compared with the diffuse background emissivity of $\sim 3 \times 10^{47} h \text{ ct s}^{-1} \text{ Mpc}^{-3}$. Thus normal galaxies contribute $\leq 1\%$.

Van Paradijs⁶ has calculated the normal galaxy contribution to the X-ray background by modelling the X-ray luminosities of galaxy constituents. He estimates 0.5–2.8% (2–10 keV) for no evolution, but investigates several ways in which this value may be increased. While we cannot address those involving evolution, our calculations indicate that further non-evolutionary effects are not important.

By using a sample of objects we have investigated emission at luminosities lower than those for which we can study individual discrete sources and have shown that normal galaxies do not apparently provide the numerous low luminosity X-ray sources which could make up the 2–60-keV diffuse background. Indeed, upper limits suggest luminosities comparable with, or a little less than, that of the Galaxy. This is consistent with the fact that the

average optical luminosity of the sample galaxies within $\sim 20 \text{ Mpc}$ is slightly lower than that of the Galaxy. An upper limit of $\sim 1\%$ of the diffuse background from such sources is derived.

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Radiolytic reduction of ceric ions in water DMSO mixtures and radioprotection

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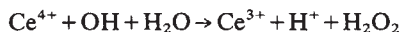
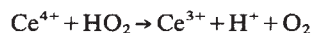
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It is convenient to classify mechanisms of radioprotection into those that involve fast radiation chemical processes and those in which the protection is the result of changes in the cell biochemistry. In the former, the added protectors have two principal effects: OH scavenging which reduces the radiosensitised damage and fast chemical repair processes involving hydrogen donation from the protector to the target radical. Typical of the first type of protector is dimethyl sulphoxide (DMSO)¹ because it is known to be a good OH scavenger ($k_{\text{OH}+\text{DMSO}} = 5 \times 10^9 \text{ mol}^{-1} \text{ s}^{-1}$, (ref. 2)). We have attempted to establish the redox properties of the OH radical adduct² as well as those of H_2O_2 or H and HO_2 radicals in water–DMSO mixtures. To this end we added to the solutions containing $0.5 \text{ M H}_2\text{SO}_4$, ceric ions at concentrations varying from 2.5×10^{-3} to $5 \times 10^{-2} \text{ M}$. The Ce^{4+} reduction yield was determined as a function of the DMSO content in the solution, by irradiating the mixture with γ rays, in the presence or absence of air. The details of the experimental procedure will be given elsewhere. We report here that the yields (G) are independent of the Ce^{4+} concentration in the range studied and of the presence or absence of air, and depend only on the DMSO concentration.

Figure 1 shows the rate of disappearance of Ce^{4+} plotted against the DMSO concentration. One sees that the $G(-\text{Ce}^{4+})$ reaches a plateau value of 17.9 ± 0.2 between 0.3 M and 1.4 M in DMSO, or 2–10% by volume. This reduction yield is very high and has never been observed previously for any solute in aqueous systems. The yield of Ce^{4+} reduction in aqueous solution is 2.5 ± 0.3 and the reduction is accompanied by O_2 formation with a yield of 0.8. In the case of deaerated water–DMSO mixtures, there is no formation of O_2 .

In acidified water, the intermediate species are H, OH and H_2O_2 , with respective yields: $G_{\text{H}} = 3.65$, $G_{\text{OH}} = 2.95$, $G_{\text{H}_2\text{O}_2} = 0.8$ (ref. 5). The reduction yield of Ce^{4+} is expressed as: $G(-\text{Ce}^{4+}) = G_{\text{H}} + 2G_{\text{H}_2\text{O}_2} - G_{\text{OH}} = 2.3$ (ref. 4). If DMSO transforms both the escaping OH radicals as well as the hydrogen peroxide precursors into reducing radicals, so that each OH

gives rise to three equivalents of reduction then the yield of reduction of Ce^{4+} may reach a value corresponding to the equation: $G(-\text{Ce}^{4+}) = G_{\text{H}} + 6 G_{\text{H}_2\text{O}_2} + 3 G_{\text{OH}} = 17.3$. The Ce^{4+} reduction reactions by these DMSO radicals would be stoichiometrically equivalent to following thermodynamically possible reactions of H_2O_2 and OH :



In these equations the yield corresponding to the capture of the radicals involved in the back reaction to form water is not taken into account. If DMSO scavenges these species the reduction yield should increase. Actually, the experimental value at the plateau is a little higher (17.9 ± 0.2) which may be the result of two simultaneous effects: an increase of the capture of the radicals coming from water which tends to increase $G(-\text{Ce}^{4+})$ and a greater participation of transitory species coming from DMSO radiolysis of which the overall yield may be smaller than that of water. This latter conjecture follows from the decrease of $G(-\text{Ce}^{4+})$ observed at DMSO concentrations from 1.4 M up to pure DMSO where the reduction yield becomes 13.9 ± 0.5 . This value is still quite large suggesting that all the intermediates coming from DMSO also reduce Ce^{4+} . This yield then corresponds to $3 G_{\text{ox}} + G_{\text{H}}$, G_{ox} being the yield of oxidising species coming from DMSO^+ created in the primary ionisation process. G_{ox} and G_{H} have been determined as equal to 3.6 and 2.1 respectively.⁶

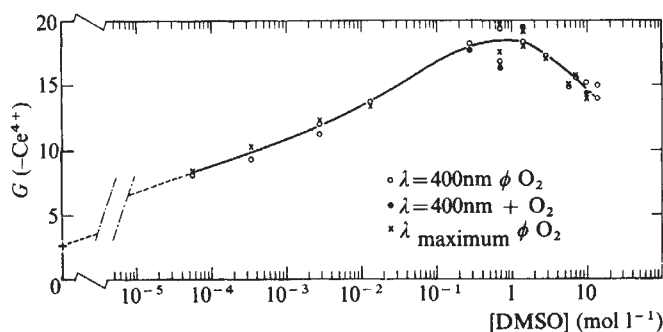


Fig. 1 The rate of disappearance of Ce^{4+} with the DMSO concentration.

It seems clear that the radicals formed by the reaction of DMSO with OH, H₂O₂, H or HO₂ all reduce Ce⁴⁺. So DMSO not only acts as a good OH scavenger, suppressing an oxidising species, but the radical formed may also have reducing properties. This latter point could be important for the use of DMSO as a radioprotector. Another important point is the experimental observation of the absence of oxygen during this reduction reaction either by H₂O₂ or by HO₂. HO₂ like OH may give an adduct with DMSO which has reducing properties, but the reaction does not form O₂. This may also increase the radioprotective properties of DMSO by suppressing a very harmful radical HO₂ and by avoiding the formation of O₂ which is itself known to be a good radiosensitiser.

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The synthesis of fluor-analogues of natural asbestos by unidirectional crystallisation

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A previous letter¹ reported a novel technique for the preparation of aligned fibrous crystals by drawing filaments of glass through a supported molten zone. The method was subsequently modified so that the filament is drawn from a melt through an orifice in the base of a conical platinum crucible². This technique has now been successfully applied to the preparation of filaments consisting of aligned fibres of fluor-amphiboles, synthetic analogues of natural amphiboles with isomorphous replacement of hydroxyl by fluorine. These aligned fibrous crystals can be compared with natural amphibole asbestos.

Considerable work has been carried out, particularly in the USSR, to synthesise fibrous asbestos³⁻⁵. The fibres produced, 80–90% fluor-amphibole, occur as a mass of matted fibres with a short brush-like growth on the surface. Fibres from the brush-like growth can be up to 3.5 cm long, although those in the bulk are very much shorter; diameters are between 0.5 and 10 μm . The dynamic nature of the present technique enables filaments of axially aligned fibres to be drawn to whatever length is desirable, limited only by the dimensions of the experimental arrangement. Moreover a greater degree of control over the crystal diameter and phase assemblage is possible by varying such parameters as melt temperature, thermal gradient, crystallisation rate (via drawing speed), nozzle diameter and hence filament diameter. Figure 1 summarises the effect of certain parameters for a single crystallising phase, which have been discussed previously². Briefly it can be seen that the temperature of crystallisation T_c decreases with increasing drawing speed V .

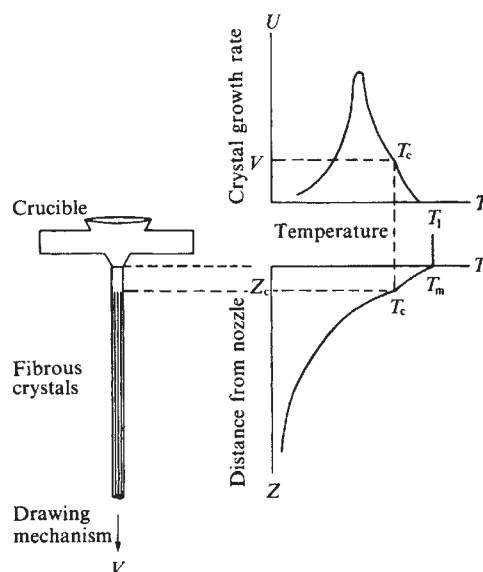


Fig. 1 Schematic diagram of apparatus for crystallisation from the melt (not to scale). Crucible lid with funnel has been omitted for clarity. T_m , T_l , T_c are melt, liquidus and crystallisation temperatures respectively; z_c , distance of crystal growth front from nozzle; V , drawing speed. Nozzle diameter is 0.9 mm and z_c is up to 1 mm.

Table 1 Batch compositions used successfully to produce fluor-amphiboles

Fluor-amphibole	Batch composition used	Liquidus	Crystalline phases in drawn filament
Fluor-magnesiorichterite $\text{Na}_2\text{MgMg}_5\text{Si}_8\text{O}_{22}\text{F}_2$	$\text{Na}_2\text{Mg}_6\text{Si}_8\text{O}_{20}\text{F}_6$	1,280 °C	(1) Water-swelling layered compound, probably fluor-tetrasilic mica ($\text{Na}_2\text{Mg}_5\text{Si}_8\text{O}_{20}\text{F}_4$) ⁹ (2) Clinoenstatite (3) Small amount of fluor-magnesiorichterite
Fluor-tremolite $\text{Ca}_2\text{Mg}_5\text{Si}_8\text{O}_{22}\text{F}_2$	$\text{Ca}_2\text{Mg}_5\text{Si}_8\text{O}_{22}\text{F}_2 \cdot \text{SiF}_4$	~1,500 °C	(1) Clinoenstatite and diopside (as two solid solutions) (2) Fluor-tremolite
Fluor-richterite $\text{Na}_2\text{CaMg}_5\text{Si}_8\text{O}_{22}\text{F}_2$	$\text{Na}_2\text{CaMg}_5\text{Si}_8\text{O}_{22}\text{F}_2 \cdot \text{Na}_2\text{SiF}_6$	1,210 °C	(1) Fluor-richterite (2) Diopside and clinoenstatite additionally in certain conditions

until the maximum crystal growth rate is reached. The temperature of the melt T_m , which is above the liquidus T_l , determines the distance of the growth front from the nozzle (z_c).

The fluor-amphiboles that we have synthesised by this method are fluor-magnesiorichterite, fluor-tremolite and fluor-richterite; chosen because X-ray and optical data are readily available^{6,7}. The first two have been produced here only in conjunction with pyroxene phases but the latter has been obtained as the only crystalline phase in the drawn filaments.

One of the main difficulties which arises when this method is applied to the synthesis of fluor-amphiboles is the loss of volatile fluorides from the melt; these are mainly SiF_4 , with some NaF if Na is present and possibly some HF by hydrolysis. To reduce loss by volatilisation, the original cell² was slightly modified by welding a platinum lid on to the conical crucible, with a narrow funnel to allow recharging, closed at the top by a removable alumina stopper. Although this does not give a gas-tight seal, it does reduce the fluorine loss considerably. Starting materials were the anhydrous compounds MgO , SiO_2 , Na_2CO_3 , CaCO_3 and MgF_2 , all of analytical purity. Batches were made up from these in proportions corresponding to the fluor-amphibole formula with a little extra fluorine and/or sodium. The most successful of the batch compositions used for each of the fluor-amphiboles is shown in Table 1, together with the crystalline phases produced and identified by powder X-ray diffraction.

In all cases where batch composition was close to the formula of fluor-magnesiorichterite the dominating phase was a water-swelling layered compound which contained twice the number

of fluorine ions per silicate group as did the amphibole, at the expense of the latter. The amount of amphibole formed falls rapidly to zero as volatilisation reduces the F and Na available. A considerable amphibole yield was obtained from melts of composition close to that of fluor-tremolite, but always accompanied by pyroxene.

Reincorporation of sodium, together with calcium, into the batch reduced the liquidus temperature so that lower melt temperatures could be used and hence loss by volatilisation reduced, giving a very much higher amphibole/pyroxene ratio in drawn filaments. In fact over most of the temperature range



Fig. 2 Optical micrograph (transmitted light, crossed polars) of filament containing fluor-richterite and some pyroxene, drawn at $11 \mu\text{m s}^{-1}$. Longitudinal section. Scale bar, $100 \mu\text{m}$.

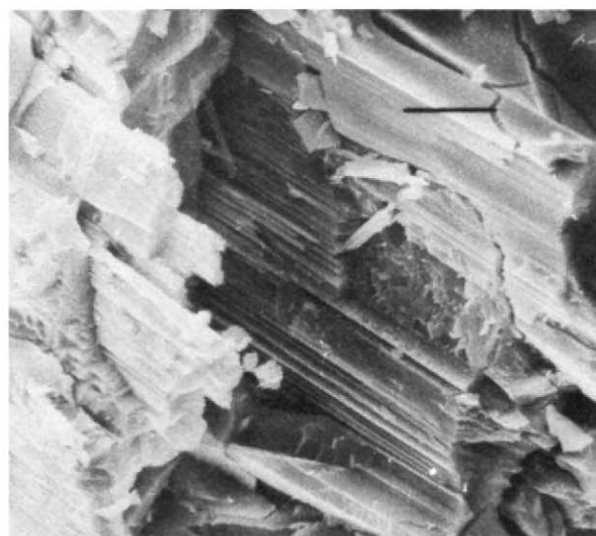


Fig. 3 Scanning electron micrograph of fracture surface of a filament containing fluor-richterite as the only crystalline phase. Scale bar, $10 \mu\text{m}$.

possible for drawing, filaments were free from pyroxene, fluor-richterite being the only crystalline phase detectable by powder X-ray diffraction. The range of melt temperatures found possible for drawing was approximately 1,200 to 1,380 °C, whilst pyroxene appeared at the start of drawing above about 1,320 °C. However, the maximum drawing speed possible for crystallisation of fluor-richterite from this melt composition is somewhat low, $15 \mu\text{m s}^{-1}$, compared with rates in excess of $60 \mu\text{m s}^{-1}$ that have been used to draw filaments of the other two fluor-amphiboles. The X-ray pattern for fluor-richterite contained a few lines not recorded by Kohn and Comefero⁷. These, however, correspond to reflections permissible by the symmetry of the crystal structure (space group $C2/m$), and are in fact recorded for some other fluor-amphiboles and/or hydroxy-amphiboles⁶⁻⁸; moreover the reflections, including the 'extra'

reflections, correspond closely in intensity and position to the pattern given by a natural richterite from Langban, Sweden, supplied by the British Museum (Natural History) which matched the data reported by Huebner and Papike⁸ for the synthetic richterite $\text{Na}_2\text{CaMg}_5\text{Si}_8\text{O}_{22}(\text{OH})_2$.

Optical examination of thin sections of filaments, between 1 and 2 mm in diameter, reveal in most cases a well aligned, polycrystalline microstructure (Fig. 2) with fibres parallel to the filament axis. The high degree of alignment was confirmed by rotation X-ray diffraction. Crystal diameters vary according to conditions used but are rather large, being typically 10–20 μm , and in the case of fluor-rich richterite filaments free from pyroxene, larger than this (~20–60 μm). Transverse sections show that the fibre crystals are of pseudo-hexagonal or rhombic skeletal cross-section with cleavage planes at angles of approximately 56° and 124° as typical of amphiboles. The residual glass is estimated from transverse sections to be approximately 10%.

Figure 3 is a scanning electron micrograph of a typical fracture surface of a fluor-rich richterite filament showing the very much finer sub-structure to these crystals, made up of laths a fraction of a micrometre thick.

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Laser observations of the ground-state hyperfine structure of sodium and of temperatures in the upper atmosphere

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The height distributions of sodium between about 80 and 100 km have been measured by several groups from observations of the resonance scattering of a tuned laser beam^{1–7}. Furthermore, estimates of temperatures in this height range have been made⁴ by incorporating a sodium cell of known temperature in the receiver of the laser radar system. In all these measurements, the linewidths of the lasers employed have been comparable with, or greater than, that of the sodium D_1 or D_2 resonance line. The ground level of sodium atoms, $3s\ ^2S_{1/2}$ consists of two hyperfine-structure components at –1.107 GHz and 0.664 GHz relative to the centroid⁸. Because of the corresponding hyperfine structure of the upper levels, $3p\ ^2P_{1/2}$ for D_1 and $3p\ ^2P_{3/2}$ for D_2 , four components contribute to the D_1 and six to the D_2 complexes. Since the relative strengths and frequencies of the ten components are well known, the theoretical line shape can be computed⁹. For temperatures above about 6 K, blending of these components occurs and for temperatures below about 456 K the line profiles of both D_1 and D_2 complexes show two distinct peaks. In this letter preliminary measurements of the profile of the D_2 line using a tunable narrow-band laser are presented and used to deduce the temperature near the peak of the sodium layer.

The effects of saturated absorption and optical pumping between the hyperfine levels of sodium atoms observed in laboratory measurements^{10,11} are expected to be negligible with

the moderate output energy (20 mJ) and the transmitter beam divergence (2.5 mrad) used.

The laser transmitter system described previously¹² has been modified by extending the resonator length and incorporating a piezo-electric etalon of 20 mm spacing to provide a tunable linewidth of about 0.1 GHz, about one-thirtieth of the widths of the D_1 or D_2 complexes at temperatures in the 80–100 km height range. The laser frequency, laser triggering, and data recording were controlled by a programmable calculator, interfaced with a digital-to-analogue converter and with 10 range-gated photon counters. The laser frequency was changed, and the photon counts were transferred to the calculator, after every laser pulse. The system was programmed to perform successive scans each of eight equally-spaced laser frequencies spanning the D_2 line; after 75 scans comprising 600 laser pulses, occupying about 10 min, the data were recorded on magnetic tape.

The first results, based on 1,200 laser pulses during between 2300 and 2330 h on the night of 30 January 1979, are presented in Fig. 1a. This shows the ratio, R_i , of the signal from the sodium layer (82–98 km) to that from a lower height range (28–30 km), which is dominated by Rayleigh scattering, with the standard error, σ_i , of this ratio for each frequency, $i = 1$ to 8. The variation of this ratio with frequency represents the corresponding variation of sodium atom cross-section. Also shown is the theoretical curve which gives the best fit to the data. Since an absolute calibration of the laser frequency was not available, this frequency was taken to be a linear function, $A + Bi$, of the frequency number, i , with two unknown parameters A and B . The theoretical curve is thus a function of four independent unknown parameters, that is

$$S_i(T, A, B, C) = C \sum_{h=1}^6 \frac{f_h}{\nu_h \sqrt{T}} \exp\{-(A + Bi - \nu_h)^2 Mc^2 / 2\nu_h^2 kT\} \quad (1)$$

where f_h and ν_h are the strength and frequency of the h th hyperfine component, M the mass of a sodium atom, c the velocity of light and k is Boltzmann's constant. The temperature T , and C , a scale factor for the ordinate in Fig. 1a, are the other two unknowns. The best fit of equation (1) to the data was obtained by minimising the χ^2 statistic given by

$$\chi^2 = \sum_{i=1}^8 \{R_i - S_i(T, A, B, C)\}^2 / \sigma_i^2 \quad (2)$$

A test of the hypothesis that each of the observed values of R_i is a random sample from a normal distribution having mean $S_i(T, A, B, C)$ and variance σ_i^2 , can be applied by determining the probability, $P(\chi^2)$, of the value of χ^2 obtained being exceeded in random sampling. The procedure for determining the means involves four nonlinear constraints, the equations

$$\frac{\partial(\chi^2)}{\partial q} = 0 \quad \text{where } q \equiv T, A, B \text{ or } C \quad (3)$$

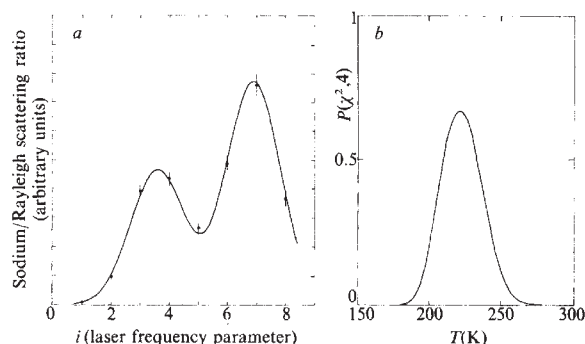


Fig. 1 a, Ratio of the sodium to Rayleigh scattered signals as a function of laser frequency for 1,200 laser shots on 30 January 1979, together with the theoretical lineshape which provides the best fit to the data. b, The probability $P(\chi^2, 4)$ as a function of temperature for the curve-fitting in a.

and hence we take the number of degrees of freedom as 4, obtaining a value $P(\chi^2, 4)$ of 0.67, indicating that the data are consistent with the theoretical relation (1).

Figure 1b shows the value of $P(\chi^2, 4)$ plotted as a function of T after χ^2 has been minimised with respect to A , B and C . The best value of T , indicated by the maximum of this curve, is (222 ± 16) K; the error estimate corresponds to one-half the half-width of the P curve at $P = 0.05$. Subsequent measurements during May 1979 indicated substantially lower temperatures, near 170 K, between 90 and 94 km. Small negative corrections can be applied to the measured temperatures after taking into account the finite laser linewidth. If $\Delta\nu_D$ represents the Doppler linewidth of each hyperfine component and $\Delta\nu_L$ is the laser linewidth, it can be shown by replacing equation (1) by its convolution with a gaussian laser line-shape function that the first-order correction is given by

$$\frac{\Delta T}{T} = -(\Delta\nu_L/\Delta\nu_D)^2 \quad (4)$$

which amounts to less than 3K for our measurements.

It is to be expected that the present method of measuring upper atmosphere temperatures will be capable of greater accuracy than that involving the use of a sodium absorption cell in the receiver which depends on integration over the spectral line shape. The accuracy currently available with the new technique can be substantially improved by the use of high laser repetition rates, improvements in receiver sensitivity and maximum photon counting rates, and optimum choice of laser frequencies. The capability of observing the small-scale structure of temperature offered by this laser technique is directly relevant to studies of the thermal balance of the upper mesosphere, of the neutral and ion chemistry involving temperature dependent rate coefficients, of noctilucent clouds and of short-wavelength atmospheric-wave disturbances. This ground-based technique could, therefore, be a useful supplement to existing satellite measurements of temperature based on observations of thermal emissions.

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Environmental fluctuation effects on the global energy balance

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Much effort has been devoted to developing simple energy-balance climatic models. Although consideration of latitudinal energy transfer^{1–4} gives more complete answers it has become clear that global, 'zero dimensional' models may also provide

much useful information^{5,6}. These models have the form:

$$C \frac{dT}{dt} = Q(1 - a(T)) - \epsilon \sigma T^4 \quad (1)$$

T is the surface temperature, C the thermal inertia coefficient, Q the solar constants, σ the Stefan constant, $a(T)$ the (generally temperature-dependent) albedo, and ϵ the emissivity of the Earth-atmosphere system. The variability of the climate system rests, therefore, on certain types of change experienced by the solar output, or by such planetary factors as emissivity, albedo, cloudiness and so forth. In addition to some long-term trends of the solar constant⁷, it has been suggested that the Sun is in an almost-intransitive state^{8,9}. Hence, it may generate large fluctuations around some mean value of its output, which will be perceived by the Earth-atmosphere system as an 'external noise' affecting Q . The fact that the terrestrial atmosphere is likely to be in an almost intransitive state¹⁰ can also generate appreciable fluctuations in factors influencing the albedo and the emissivity. In the absence of precise knowledge of the mechanism of these fluctuations, one is again tempted to regard them as an 'external noise' affecting $a(T)$ and ϵ . We explore here the qualitative effect of such environmental fluctuations in the thermal regime¹³, at the level of a zero-dimensional planetary model. Previous analyses of nonlinear systems of chemical and biological interest^{11,12} have shown that external noise can dramatically affect the macroscopic behaviour predicted by the deterministic equations of evolution, if coupled to these equations in a multiplicative way.

All cases considered here refer to such multiplicative coupling. In this respect, our analysis differs from the work of Lemke¹⁴ and Fraedrich⁵ which is concerned primarily with additive fluctuations. Although such fluctuations are important, clearly the multiplicative coupling is a more appropriate representation of the dynamics of a complex system such as the Earth-atmosphere.

From the mathematical point of view, the effects of external noise change equation (1) to a stochastic differential equation, to which the Itô calculus can be applied¹⁵. Let us adopt an abstract notation and decompose equation (1) into two pieces, $f(T)$ and $g(T)$, which are linearly coupled through some parameter λ

$$\frac{dT}{dt} = f(T) + \lambda g(T) \quad (2)$$

Let λ be subject to environmental fluctuations referred to above. For simplicity, we take the idealised picture of a gaussian white noise. (The possible shortcomings of white noise, related to the possibility that the fluctuating parameters reach negative values, means that in each case the nature of the boundaries of the stochastic process must be checked carefully.) Hence¹⁵:

$$\lambda = \bar{\lambda}(1 + \xi_t)$$

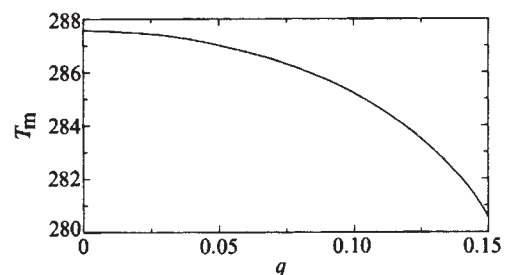


Fig. 1 Dependence of most probable temperature T_m on variance q . Q fluctuates around the mean value of $1.95 \text{ cal cm}^{-2} \text{ min}^{-1}$. The thermal inertia coefficient used in the simulation is $C = 7.5 \text{ kcal cm}^{-2} \text{ K}^{-1}$. The emissivity ϵ is kept at the value 0.6, and the albedo is $a = \alpha + \beta T$ where $\alpha = 2.7888$, $\beta = -0.0086$.

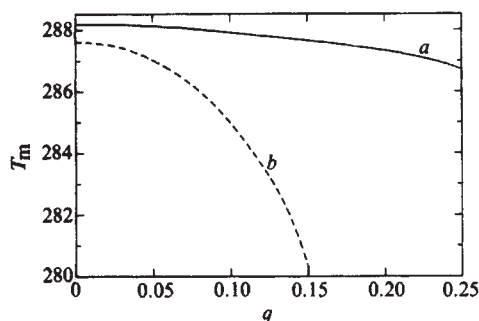


Fig. 2 T_m versus q when ε fluctuates around the value of 0.6. The thermal inertia coefficient is as in Fig. 1. *a*, The albedo is taken to be constant, $\bar{a} = 0.31$. *b*, The albedo is described by $a = \alpha + \beta T$ where $\alpha = 2.788$, $\beta = -0.0086$.

where $\bar{\lambda}$ is the value of λ appearing in the deterministic description and ξ_t is the derivative of a Wiener process, W_t :

$$\begin{aligned} \langle \xi_t \rangle &= 0 \\ \langle \xi_t \xi_{t'} \rangle &= q^2 \delta(t - t') \end{aligned} \quad (3)$$

Equation (2) then takes the form (we now use the Itô rather than the Stratonovich interpretation¹⁵):

$$dT = [f(T) + \bar{\lambda}g(T)] dt + q\bar{\lambda}g(T) dW_t \quad (4)$$

which is equivalent to a Fokker-Planck equation for the probability distribution $P(T, t)$:

$$\begin{aligned} \frac{\partial P}{\partial t} &= -\frac{\partial}{\partial T} [f(T) + \bar{\lambda}g(T)]P(T, t) + \\ &+ \frac{1}{2}q^2 \frac{\partial^2}{\partial T^2} \bar{\lambda}^2 g^2(T)P(T, t) \end{aligned} \quad (5)$$

A macroscopically observable stable temperature clearly corresponds to a maximum of the steady-state distribution $P_{st}(T)$ which obeys the equation:

$$-[f(T_m) + \bar{\lambda}g(T_m)] + \frac{q^2}{2} \frac{d}{dT} [\bar{\lambda}^2 g^2(T_m)] = 0 \quad (6)$$

we now apply this relation to the following situations.

(1) Q fluctuates around the present-day value of $1.95 \text{ cal cm}^{-2} \text{ min}^{-1}$. If the planetary albedo, a , is taken to be constant, the noise becomes additive and equation (6) gives $dg^2/dT=0$; hence one obtains the usual value of planetary temperature corresponding to present-day conditions. On the other hand, the fluctuations of Q begin to be effective if the planetary albedo depends on T . The simplest non-trivial such dependence is given by the linear function $a(T) = \alpha + \beta T$. Such expressions have been used widely^{5,6,16}, at least in the range between the highest temperature than can exist when the whole Earth is covered with ice, and the lowest possible temperature when no ice is present. Note that some pathological properties of linear albedo-temperature feedback pointed out by Schneider and Gal-Chen for one-dimensional energy-balance models¹⁷ are not necessarily reproduced in a zero-dimensional planetary model. In the latter, the albedo must depend on the planetary temperature in a continuous fashion at least in a range of temperatures not too remote from present-day conditions.

(2) ε fluctuates around some mean value $\bar{\varepsilon}$ corresponding to a surface temperature of 287.6 K in the absence of fluctuations. The planetary albedo is taken to be, successively, equal to a constant value $\bar{a}$ or to a linear function of T . Physically speaking, fluctuations of ε at fixed albedo could be attributed to random modifications of atmospheric composition.

(3) a and ε both follow the fluctuations of cloudiness, n . The parameterisations of a and ε in terms of n are taken from Cess¹⁶ and Schneider¹⁸, and

$$n = 0.5(1 + \xi_t) \quad (7)$$

We now describe the results of some representative numerical simulations.

Figure 1 shows T_m as a function of q , the square root of the variance of Q . As q increases from 0.005 to 0.05 the most probable temperature decreases from about 287.6 K to 287 K. For higher q the decrease of T_m is more pronounced.

Finally, when $q \geq 0.16$ then T_m drops to extremely low values. The precise numbers are of no importance here, as the model should not be valid anyway for such ranges of temperature and additional feedback mechanisms could take over. The main point therefore of Fig. 1 is the tendency for runaway to lower temperature values.

Curve *b* of Figure 2 shows the analogous result in terms of the variance of ε for a linear albedo-temperature feedback (note that in this case equation (6) is an equation of seventh degree in T_m —a degree higher than that of the deterministic equation). Again, for $q \geq 0.16$ there is a similar tendency of runaway to extremely low values of T_m . Curve *a* describes the situation in the constant albedo case: the same tendency to lower T is observed, although there seems to be no runaway effect.

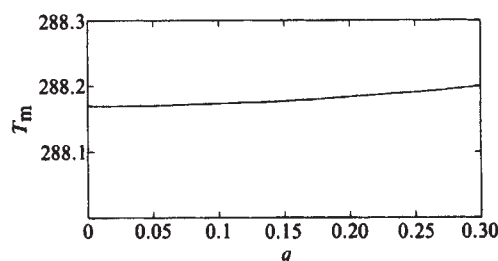


Fig. 3 Effect of fluctuating cloudiness using the following parameterisations. For the albedo (see ref. 16): $a = 0.18 + 0.26n$. For the emissivity (see ref. 18, for an effective cloud height of about 6.5 km): $\varepsilon = 0.71 - 0.22n$.

The effect of fluctuating cloudiness is rather different and described in Fig. 3 (here again, the equation for T is of seventh degree). As q increases from 0 to 0.30, the temperature remains substantially the same, varying by less than one-tenth of a degree. It appears that the fluctuations have opposite effects on the input and output terms in equation (4), thus giving a small overall effect. Another cause of the smallness of the effect may be that in the parameterisation of equation (7), cloudiness is taken to be temperature independent.

The thermal regime in a fluctuating environment seems thus to be established at temperatures that may differ substantially from those corresponding to a constant environment (actually, there always seems to be a tendency towards decreasing values). Eventually, in the presence of giant fluctuations a runaway regime may be reached in which there cannot be a stationary solution in a physically reasonable temperature range. These unexpected phenomena are all due to the multiplicative coupling between fluctuating parameters and the temperature¹¹. In the case of additive noise or of linearised response^{5,14} the effect would vanish because the function g^2 in equation (6) would be constant. One would thus recover the solution of the deterministic rate equation. It would be interesting to extend the present investigation to more realistic types of noise and to conditions prevailing in other planets, where a runaway regime is believed to exist.

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Intercalation of amino acids and polypeptides into 2H-TaS₂

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2H-TaS₂ can be intercalated by organic amines and empirical rules have been formulated¹⁻³ governing the properties that chemical species must possess in order to intercalate. One of these rules is that molecules in which the nitrogen is basic ($pK_a > 4$) usually form stable compounds provided the amine does not have bulky substituents. For certain amino acids, the values of pK_a are significantly larger than this critical value (for example, glycine $pK_2 = 9.78$)⁵ so that if the zwitterion is in a state where it can function as a base, it might be expected that the amino acid would intercalate 2H-TaS₂. This prediction has been verified for a number of amino acids. The discovery of these intercalation reactions provides a new range of intercalated compounds for study and also opens up the possibility of *in situ* polymerisation.

Saturated solutions of amino acids (chromatographically homogeneous or laboratory grade purity) in distilled water were prepared in a borosilicate glass ampoule at 75 °C. Samples of about 0.15 g of 2H-TaS₂, in either powder or single crystal form, were added and the ampoule was then sealed in air. The tubes were placed in an oven at 100 °C for between 1 and 3 weeks.

The intercalation reaction was monitored by opening capsules sequentially and examining the powder on an X-ray

diffractometer with CuK α radiation. When the reaction product was entirely the stage 1 compound (judged by the position and line breadth of the 00 l reflections), the new interlayer separation was obtained by extrapolating the parameter estimated from individual 00 l reflections to $\theta = 90^\circ$ as a function of $\cot \theta \cos \theta$. Selected single crystals were also examined using a single crystal goniometer with MoK α radiation.

The composition x in the formula TaS₂(amino acid) _{x} was determined using a Perkin-Elmer thermobalance. In making this calculation, the entire weight loss was attributed to amino acid for there was no sudden change in the rate of weight loss as might be expected if water was also intercalated along with the amino acid.

Superconducting transition temperatures were measured using a lock-in variable impedance method. Temperatures were measured using an Allen-Bradley carbon resistance thermometer calibrated against the vapour pressure of liquid helium and the superconducting transition temperatures of lead and niobium as calibration points. As noted in Table 1, certain compounds did not exhibit a superconducting transition between 1.2 and 15 K.

The intercalated species are shown in Table 1 and include amino acids such as glycine, β -alanine, L-proline, picolinic acid, diketopiperazine (glycine anhydride) as well as the polypeptides of glycine to pentaglycine. Attempts to intercalate isonicotinic acid, DL-proline and L-hydroxyproline were not successful. Values of the expanded gap (the difference between the layer separation before and after intercalation) show that, except for valine, the molecules lie parallel to the TaS₂ layer with the p_z orbital containing the nitrogen lone pair orientated perpendicular to the layer. The results indicate that the peptide bond actively promotes intercalation. Thus glycine anhydride does not possess any amino groups at all and intercalation must have involved the two ring nitrogen atoms. Moreover pentaglycine (a 16-atom chain excluding hydrogens) remains parallel to the layer with an expanded gap of 3.4 Å in contrast to pentadecylamine¹ (also a 16-atom chain) which lies perpendicular to the layer with a 39 Å expanded gap.

Single-crystal X-ray studies indicate that the a -axis orientation of the matrix crystal is preserved on intercalation although the layer stacking order is destroyed (only hk rods are observed). This suggests a random sequence of stacking faults in contrast to the regular sequence of faults found in 2H-TaS₂(pyridine)⁴. Possibly the present choice of reaction conditions does not favour a highly orientated reaction product.

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Table 1 Amino acids and polypeptides intercalating with 2H-TaS₂

Formula	Substance	pK_2^*	Aq. conc. (g ml ⁻¹)†	$x \ddagger$	Interlayer § separation (Å)	Expanded gap (Å)¶	Superconductivity	
							T onset (K)	Width (K)
NH ₂ .CH ₂ .COOH	Glycine	9.78	0.25	0.289	9.71 (0.009)	3.67	3.4	1.1
NH ₂ .(CH ₂ .CO.NH) _{$n-1$} .CH ₂ .COOH	Diglycine $n = 2$	8.25	0.125	0.172	9.43 (0.016)	3.39	2.6	0.6
	Triglycine $n = 3$	8.09	0.125	0.085	9.40 (0.017)	3.36	4.2	1.4
	Tetraglycine $n = 4$		0.036	0.076	9.4 (0.04)	3.4	No transition detected between 1.2 and 1.5 K	
	Pentaglycine $n = 5$		0.005	0.066	9.4 (0.04)	3.4		
CO.NH.CH ₂ .CO.NH.CH ₂	Glycine anhydride (diketopiperazine)		0.080	0.158	9.5 (0.05)	3.5	2.8	0.8
CH.CH.CH.CH.N.C.COOH	Picolinic acid		0.200	0.163	9.4 (0.03)	3.4	3.4	1.4
CH.CH.CH.N.CH.C.COOH	Nicotinic acid		0.166	0.165	9.56 (0.005)	3.52	3.6	1.8
CH(CH ₂) ₂ .CH(NH ₂).COOH	Valine	9.72	0.100	0.255	12.6 (0.07)	6.6	No transition detected between 1.2 and 15 K	
NH ₂ .CH ₂ .CH ₂ .COOH	β -Alanine		0.050	0.219	9.4 (0.04)	3.4	2.5	0.5
CH ₂ .CH ₂ .CH ₂ .NH.CH.COOH	L-Proline		0.400	0.181	10.00 (0.014)	3.96	4.7	2.7

* From ref. 5.

† Aqueous concentration: this solution was allowed to react for 7 days at 95 °C.

‡ Composition TaS₂(amino acid) _{x} .

§ The e.s.d. is given in parenthesis.

¶ The difference between the interlayer separation before and after intercalation.

The superconducting transition temperatures are generally lower than for the amine intercalates¹ suggesting that the density of states at the Fermi level is modified less than in the amines. No superconducting transition could be detected in three of the compounds. The result was unexpected and further study is necessary before we can comment on the significance of this finding.

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Effect of *in situ* leaching on amino acid racemisation rates in fossil bone

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Amino acid racemisation reactions provide a promising approach for estimating the absolute ages of fossil bones¹. However, an important question remains unanswered: what is the effect of *in situ* leaching on apparent racemisation rates^{2,3}? We report here the relationship between leaching history and the corresponding D/L aspartic acid ratio for 12 well-dated fossil samples, using γ -carboxyglutamic acid (Gla) as a quantitative index of leaching⁴, in an attempt to resolve this problem.

High-temperature simulation studies provided the initial evidence that intensive leaching of bone organic matrix can substantially change the D/L ratios of the matrix amino acids and consequently generate misleading apparent ages^{2,3}. In contrast, racemisation ages of natural fossil samples (presumably representing a range of different leaching histories) were found to be in close agreement with independently determined radiocarbon dates^{5–7}. Availability of a bone-specific leaching index (Gla) unaffected by environmental organic matter⁴ has allowed us to study directly the possible role of leaching in the alteration of the D/L ratios used for the amino acid dating of fossil bones.

The six pairs of fossil bone samples in which Gla was measured included a wide range of ages, environmental temperatures, and leaching histories (Table 1). The first two samples (Buhen Horse and Mindanao SU-1) came from localities having nearly identical air temperatures. However, their respective environments were quite different in terms of annual rainfall—the Buhen Horse sample was from the arid Sudan (0.4 cm yr⁻¹ precipitation) and the Mindanao SU-1 sample was from an estuarine swamp in the Philippines (232 cm yr⁻¹ precipitation)—and this is reflected by their Gla values of 845 and 516 nmol g⁻¹, respectively. As percentages of the Gla concentration in fresh bovine bone (1,076 nmol per g bone⁴), the Gla content of the Buhen Horse sample is 79% and the Mindanao sample 48%. Both samples have the collagenous organic matrices expected in slightly to moderately leached specimens². Despite a significant difference in leaching histories

reflected by the Gla contents, the two samples show essentially equal racemisation rate constants (k_{asp}) when the environmental temperatures are taken into account.

Both of the La Jolla palaeo-Indian samples have undergone substantial leaching as shown by their low Gla values. Although sample SDM 16709 has a Gla content equivalent to only 5% of fresh bovine, its organic matrix is still collagenous in composition. When the D/L ratio of SDM 16709 is used to calibrate the racemisation reaction, the resulting racemisation age for W-12/76 is in good agreement with the radiocarbon date.

The sites at which the Olduvai Gorge and Nasera Rock Shelter samples were collected are only about 30 km apart. Both have been heavily leached, although the less intensely leached Olduvai Gorge sample (13% Gla content relative to fresh bovine bone) still has a collagen-like pattern. The calculated racemisation age of the Nasera Rock sample (using the Olduvai Gorge specimen for calibration) is within 8% of its radiocarbon date.

The Murray Springs and Double Adobe samples, very similar in age, are also intensively leached as shown by the Gla contents and the absence of collagen in both organic matrices. As a result of good agreement between the D/L aspartic acid ratios of the Double Adobe sample and the calibrating sample (Murray Springs), the calculated racemisation age is concordant with the radiocarbon age.

The two older samples from the Nasera Rock Shelter (levels 5A and 6) have similar radiocarbon ages. Both have undergone severe leaching and neither show detectable Gla nor a collagenous organic matrix. The racemisation age of level 6, based on a calibration with level 5A, is about 13% higher than the radiocarbon age.

The oldest pair of samples is represented by two from Mt Carmel. Both are leached to an extreme degree as shown by the absence of Gla in the Tabun sample (non-collagenous) and the trace of Gla in the Sefunim specimen (collagenous). Although there is an age difference of ~14,000 yr between the Sefunim calibration sample and the Tabun sample, the calculated racemisation age for the Tabun specimen is within 8% of the radiocarbon age.

The data in Table 1 indicates concordance of aspartic acid racemisation age with radiocarbon data for each of the six unknown samples (despite measurable differences in leaching history between the unknown and calibration samples). Thus, *in situ* leaching seems to have a negligible effect on the racemisation parameters calculated from these six pairs of fossil bone samples.

How can this finding be reconciled with the original laboratory results showing significant alteration of racemisation rates with intensive leaching^{2,3}? A closer examination of the

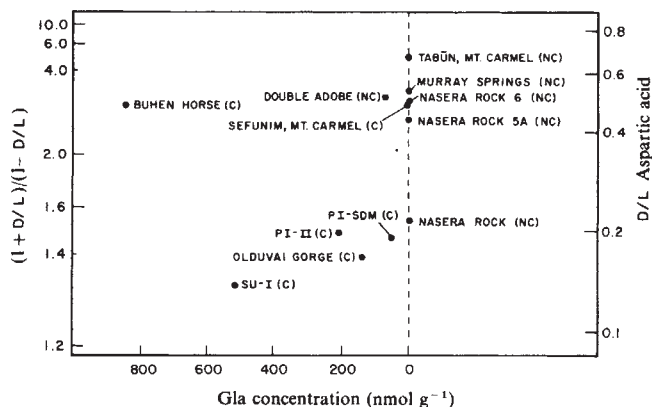


Fig. 1 Relationship between aspartic acid racemisation parameters and degree of *in situ* leaching. Intensity of leaching increases with decreasing values of Gla content. The parameter $\ln[(1 + D/L)/(1 - D/L)]$ is the key variable in the equation used to calculate racemisation ages⁸. C, collagenous matrix; NC, non-collagenous matrix.

leaching data (Fig. 1) suggests an answer to this question and helps to define the conditions in which leaching could adversely affect racemisation calculations.

The 12 samples segregate into two major clusters (Fig. 1): a low aspartic acid D/L ratio group ($\bar{X} = 0.18$), and a high aspartic acid D/L ratio group ($\bar{X} = 0.51$). The lower cluster of five samples can be characterised as young in age ($\bar{X} = 4,100$ yr), moderately leached (only one sample has < 0.1 nmol Gla per g), and predominantly collagenous in composition (only one sample contains a non-collagenous organic matrix). In contrast, the other group of seven samples with higher D/L values has a mean age of 12,400 yr, generally exhibits intense leaching (five samples have undetectable or trace amounts of Gla) and contains predominantly non-collagenous matrices (only two samples display a collagen pattern).

Note that amino acid concentration (Table 1), another possible leaching index, generates a plot remarkably similar to the Gla plot (Fig. 1). With the exception of the Buhen Horse/Mindanao comparison, the sample having the higher amino acid concentration in each pair also had the higher Gla content. However, the Mindanao sample, with a 28% higher amino acid concentration than the Buhen Horse specimen, showed a 39% lower Gla content. This unusual relationship is thought to result from the interaction of two factors: (1) minimal solubilisation (through decreased chain length) of the collagen in the Mindanao sample during the short time interval represented

(~500 yr); and (2) a more rapid loss of Gla in the Mindanao sample due to the intensive leaching conditions of its estuarine environment. Although more susceptible to perturbation by environmental contamination, amino acid concentration could be used as a leaching parameter in very heavily leached samples after Gla has disappeared.

It is probably significant that, with only one exception (the slightly leached Buhen Horse specimen), the sample used to calibrate the racemisation reaction is in the same group as the unknown sample to be dated. This suggests the possibility that each of the calibration samples has, in effect, calibrated the racemisation reaction in the corresponding unknown for degree of leaching as well as environmental temperature. This hypothesis may be tested by attempting to 'cross-calibrate' between the two groups using data from the Nasera Rock Shelter site (Table 1). If the k_{asp} value ($7.4 \times 10^{-5} \text{ yr}^{-1}$) used for the young (~2,000 yr BP) Nasera Rock Shelter sample (low D/L group) is used to calibrate the racemisation process for dating the Nasera Rock Shelter Level 6 sample (high D/L group), the resulting age estimate is 6,200 yr BP or 73% lower than the radiocarbon age. In addition, if the k_{asp} value of the calibration sample (post-glacial exposure) is reduced by 40% to compensate for the lower integrated temperature of the older Nasera Rock Shelter (Level 6) sample (postglacial and glacial exposures)⁹, the calculated amino acid racemisation age is only increased to 10,400 yr BP (55% less than the radiocarbon age). As shown in Table 1, the

Table 1 Effect of *in situ* leaching on amino acid parameters in fossil bones

Site and description	Mean annual air temperature	D/L Aspartic acid*	k_{asp} (per yr)	Racemisation age (yr)†	Radiocarbon (yr) or historical age	Gla content (nmol g ⁻¹)‡	Amino acid concentration (nmol g ⁻¹)§	Organic matrix¶
Wadi Halfa, Sudan	26°C	0.474	1.21×10^{-4}	—	1,675 BC	845(11.8)	1,192,000	C
Buhen horse								
Mindanao, Philippines	27°C	0.136	1.48×10^{-4}	—	AD 15–16th century	516(11.7)	1,522,000	C
SU-1 (Butnan City)								
Near La Jolla California								
Palaeo-Indian skeletons								
SDM 16709 (PI-SDM)	16°C	0.189	1.45×10^{-5}	—	8,360 ± 75 (Pta-1712)	51(0.6)	81,150	C
W-12/76 No. II (PI-II)	16°C	0.195	—	8,800	8,330 ± 160 (Pta-1809)	211(3.9)	381,600	C
Olduvai Gorge, Tanzania	25°C	0.165	7.4×10^{-5}	—	1,390 ± 40 (LJ-2979) 1,330 ± 100 (LJ-3330)	144(1.0)	424,400	C
Nasera Rock Shelter, Tanzania (~30 km from Olduvai)	25°C	0.212	—	1,960	2,060 ± 100 (ISGS-438) 2,180 ± 200 (ISGS-438)	< 0.1	4,292	NC
Murray Springs, Arizona, nos 23–51	17°C	0.520	4.5×10^{-5}	—	11,230 ± 340 (A-805)	< 0.1	1,685	NC
Double Adobe, Arizona, nos 15–45	17°C	0.500	—	10,600	10,420 ± 100 (A-1152)	70(1.2)	3,103	NC
Nasera Rock Shelter, Tanzania								
Level 5A	25°C	0.428	1.81×10^{-5}	—	21,600 ± 400 (ISGS-445)	< 0.1	1,814	NC
Level 6	25°C	0.486	—	26,000	22,900 ± 400 (ISGS-425)	< 0.1	1,182	NC
Mt. Carmel, Israel								
Sefunim layer 8–9	19°C	0.472	1.64×10^{-5}	—	~ 27,000 Upper Paleolithic	4(0.4)	7,344	C
Mt Carmel, Israel								
Tabūn lower layer C	19°C	0.658	—	44,000	40,900 ± 1,000 (GrN-2729)	< 0.1	1,430	NC

* All samples hydrolysed with 6M HCl for 24 h D/L. Aspartic acid ratios were determined as described elsewhere^{7,8}.

† Calculated by using the k_{asp} of the other pair member as the calibration sample as previously described⁷.

‡ Mean values and standard deviations (in parentheses) for duplicate analyses of single fossil samples. Alkaline hydrolysis and Gla analysis of each 2-mg sample were conducted as previously described⁴. The Gla content of fresh bovine cortical bone is about 1,100 nmol g⁻¹ (ref. 4).

§ Summation of 12 individual amino acid concentrations (Asp, Thr, Ser, Glu, Gly, Ala, Val, allo-Ileu, Ileu, Leu, Tyr, Phe).

¶ C, collagenous; NC, non-collagenous. Determined by amino acid analysis of acid hydrolysates.

racemisation age for this same sample calibrated with the Nasera Rock Shelter Level 5A sample (same high D/L group) is 26,000 yr BP, 13% higher than the radiocarbon date. This example further suggests that a 'cross-over' between the two groups in calibrating a racemisation reaction may introduce substantial error in estimating absolute age.

The need for intra-group calibration most probably stems from key compositional differences between the two groups produced, in part at least, by *in situ* leaching. Although detailed information on the relevant properties (for example, molecular weight, amino acid composition, and degree of racemisation) of each individual matrix component is not yet available, comparative total amino acid compositions provide some evidence for a requirement of compositional similarity between calibration and unknown samples. As shown in Table 1, four of the six racemisation calculations involved sample pairs having organic matrices of similar composition (2 collagenous; two non-collagenous). Note that the only successful cross-calibration between the two groups involves one of the compositionally similar pairs—the collagenous Buhen Horse and Mindanao SU-1 samples. These two samples also show the lowest degrees of leaching among the samples analysed. Successful calibration with pairs of similar organic matrices is not unexpected as the laboratory leaching data indicates changes in racemisation rates coincident with the compositional change from collagenous to non-collagenous matrix¹⁰.

The remaining two pairs each involved a combination of different matrices—Olduvai Gorge (collagenous)/Nasera Rock Shelter (non-collagenous) and Sefunim (collagenous)/Tabūn (non-collagenous). An explanation for successfully achieving concordancy with these unlike matrices will require detailed characterisation of the major diagenetic components of each matrix to reveal key compositional and stereoisomeric similarities not evident in overall amino acid composition. Nevertheless, it is interesting that the calibration and unknown samples of each pair are in the same D/L group (Fig. 1) despite their dissimilar compositions.

No significant alteration in racemisation rates by *in situ* leaching was detected in this study of six pairs of fossil bone samples. The data (Fig. 1), however, suggest that the general concordancy observed between racemisation ages and radiocarbon dates may have resulted from a close matching of the calibration and unknown samples in each pair in terms of composition, leaching history and degree of racemisation. Accordingly, we recommend that amino acid composition, Glu content and amino acid concentration be measured with the D/L ratio in both the calibration and unknown samples to minimise the possibility of introducing significant errors in the calibration step. In addition to improving the reliability of the racemisation dating method, the routine measurement and reporting of these parameters would, over a period of time, generate a valuable data base for profiling the leaching-racemisation relationship at ambient temperatures.

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Origin and palaeoenvironmental interpretation of sarsens

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No detailed explanation has yet been provided for the origin of sarsens (silicified Cenozoic sediments) which are widely distributed across southern England (Fig. 1). The problem of sarsen formation is related to that of analogous terrestrial siliceous deposits recorded from every continent except Antarctica^{1–7}. In addition sarsens are of considerable archaeological interest as they were extensively used in the construction of megalithic monuments, notably those at Avebury and Stonehenge. It has been suggested that sarsens are glacially transported erratics^{8,9}, although this view has been challenged¹⁰. Their occurrence in areas in which other evidence of glacial activity is lacking, and the preservation on the surface of some sarsen stones of pockets of very weakly cemented sand, does not concur with the idea of long-distance transportation, but rather suggests local derivation from an original Cenozoic cover. Here the initial formation of sarsens is considered and recognition that they represent silcrete remnants enables broad conclusions to be drawn about their genesis and palaeoenvironmental significance. From macromorphological, micromorphological and chemical comparisons with low latitude silcretes, primarily from southern Africa, it is concluded that sarsens represent remnants of surface and near-surface silicification developed on tectonically stable landsurfaces of minimal local relief. Most sarsens seem to have formed under a semi-arid or arid climate, although there is evidence of development in a relatively humid environment for some occurrences.

As there are no entirely reliable reports of *in situ* sarsen occurrences, field evidence is confined to individual sarsen stones, up to 5 m or more across, found resting on the Chalk and on, or within, Cenozoic sediments. Sarsens range from orthoquartzites to predominantly fine-grained siliceous sediments and silicified conglomerates (Hertfordshire and Bradenham Puddingstones). A surface, or near-surface, origin is indicated by the presence of apparent rootlet channels in many samples and, more rarely, by silicified roots¹¹. Sarsens represent various forms of silcrete¹² and consequently their genesis may be elucidated by comparison with the extensive silcrete occurrences of Africa and Australia.

Sarsens are highly siliceous sediments (Table 1). Micromorphologically they can be classified into GS-(grain-supported), F-(floating) and C-(conglomeratic) fabric types¹³. GS-fabric sarsens consist of skeletal framework grains (>30 µm across), almost exclusively of quartz, usually cemented by optically continuous overgrowths, and less frequently by microquartz. F-fabric sarsens are composed of predominantly quartz skeletal grains, floating in a microquartz or cryptocrystalline silica matrix. C-fabric sarsens most commonly consist of flint pebbles in a 'matrix' of the GS-fabric type.

Field investigations of silcretes from southern Africa have shown that a distinction can be made between those occurrences associated with weathering profiles (weathering profile silcretes, WPS), and those that are not (non-weathering profile silcretes, N-WPS). These differences in field occurrence are accompanied by contrasting chemical and micromorphological characteristics. GS-fabrics, C-fabrics, optically continuous overgrowths, chalcedonic overgrowths and chalcedony vugh-fills are absent in WPS while M-(matrix) fabrics (<5% skeletal grains (>30 µm across)) are abundant (Table 2). In N-WPS authigenic glauabules and colloform features are apparently absent and M-fabrics are rare. WPS are titanium-rich (TiO₂ > 1%) while N-WPS are titanium-poor (TiO₂ < 0.2%) (Table 1). N-WPS are typically

Table 1 Bulk chemical analyses of sarsens and southern African silcretes

	SiO ₂	Al ₂ O ₃	TiO ₂	Fe ₂ O ₃	MgO	CaO	K ₂ O	MnO	P ₂ O ₅	Ignition loss
Mean composition of 51 WPS from southern Africa	94.70	0.71	1.82	1.34	0.13	0.10	0.02	0.01	0.04	1.16
Mean composition of 13 N-WPS from southern Africa	93.92	1.43	0.13	0.66	0.55	0.12	0.68	0.07	0.03	2.41
GS-fabric sarsen with optically continuous overgrowths (A)	99.56	0.22	0.07	<0.01	—	<0.01	—	<0.01	<0.01	0.14
GS-fabric sarsen with optically continuous overgrowths (B)	98.79	0.15	0.03	—	0.47	0.02	—	0.01	—	0.54
F-fabric sarsen (C)	96.12	0.29	2.08	0.80	—	0.03	—	<0.01	0.02	0.66
C-fabric sarsen (D)	98.56	0.20	0.16	0.07	—	0.02	—	<0.01	0.02	0.96
C-fabric sarsen (E)	97.90	0.24	0.02	0.54	0.37	0.02	—	<0.01	0.04	0.88

Analyses by XRF normalised to 100%. The high mean K₂O content of N-WPS is associated with (glauconite-rich?) green pan silcretes (silicified pan clays). Letters in parentheses refer to sample locations in Fig. 1.

more chemically and micromorphologically variable than WPS as they represent the silicification of a wide variety of host materials including calcretes, fluvial deposits and pan sediments.

In the sarsen samples examined the GS-fabric type (with optically continuous overgrowths) is the most common. F-fabrics are relatively scarce, except locally. Comparative micromorphological evidence from southern African silcretes militates against a weathering profile origin for GS- and C-fabric sarsens (Table 2). The status of F-fabric sarsens is less certain in that the micromorphological and chemical evidence points to a weathering profile origin for at least some occurrences. This is supported by the high titanium content (2.08% TiO₂) (Table 1) of an F-fabric sarsen which is micromorphologically identical to many F-fabric WPS from southern Africa (Fig. 2). The titanium appears to be present in dull brownish aggregates (identified by X-ray diffraction as anatase in southern African silcretes) within the matrix. Titanium- and iron-rich zones as colloform features have also been reported in F-fabric sarsens (K. Isaac, personal communication). Further analyses are required to establish whether F-fabric sarsens are characteristically titanium-rich.

The nature of the host material and of the circulating solutions responsible for silicification are the key factors in influencing sarsen micromorphology. GS-fabric sarsens formed through passive void-filling by overgrowths or microquartz of a quartzose host sediment. The development of optically continuous overgrowths implies a lack of clay within the host sediment^{14,15} and circulating solutions characterised by both low concentrations of silica and of other ions (which could disrupt the orderly growth of macro-crystals). Conversely the presence of

intergranular clay and of relatively high concentrations of silica and/or 'disruptive' ions would promote microquartz or even cryptocrystalline silica precipitation¹⁶. A similar interpretation may be applied to C-fabric sarsens as these differ from the GS-fabric type only in the size range of their skeletal grain fraction. F-fabrics can be generated by displacement or partial replacement of skeletal grains or by replacement of the matrix of an F-fabric host material such as clayey weathering deposit containing floating quartz grains. Matrix replacement is the favoured interpretation for sarsens as evidence of marked skeletal grain replacement is lacking and as displacive growth of authigenic quartz within voids is an untenable explanation of F-fabrics with a small skeletal grain component (Fig. 2) because of the considerable volume increase required. In silcretes from southern Africa there is no field evidence for the anticipated macroscale expansion which would be associated with F-fabrics generated by displacive crystal growth (such as there is in some cases for calcretes in the form of pseudoanticlines).

Previous explanations of sarsen formation have focused on macroscale climatic factors and have largely ignored geochemical conditions and micromorphological properties. A capillary rise mechanism has been favoured involving silica mobilisation and surface concentration (due to evaporation) in response to a seasonally dry climate¹¹. However, only thin surface crusts (rather than the typically observed short-axis sarsen dimensions of ~1 m) could form in this way as initial surficial silica precipitation would inhibit, and eventually prevent, the upward movement of solutions to the surface.

Comparison with silcretes from southern Africa and Australia

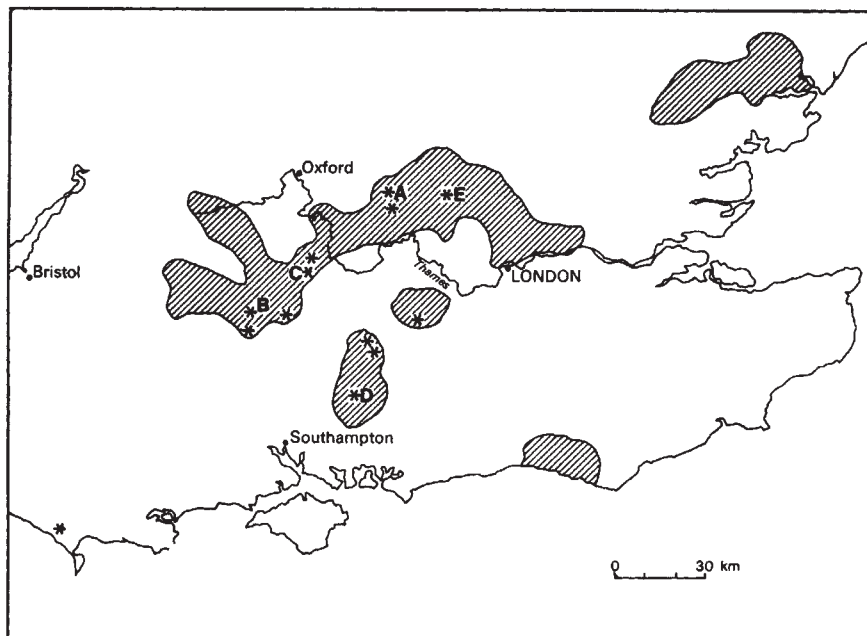


Fig. 1 Distribution of sarsens in southern England. Shaded areas indicate main regions of sarsen concentrations. Sample sites marked by asterisk. For key to letters see Table 1.

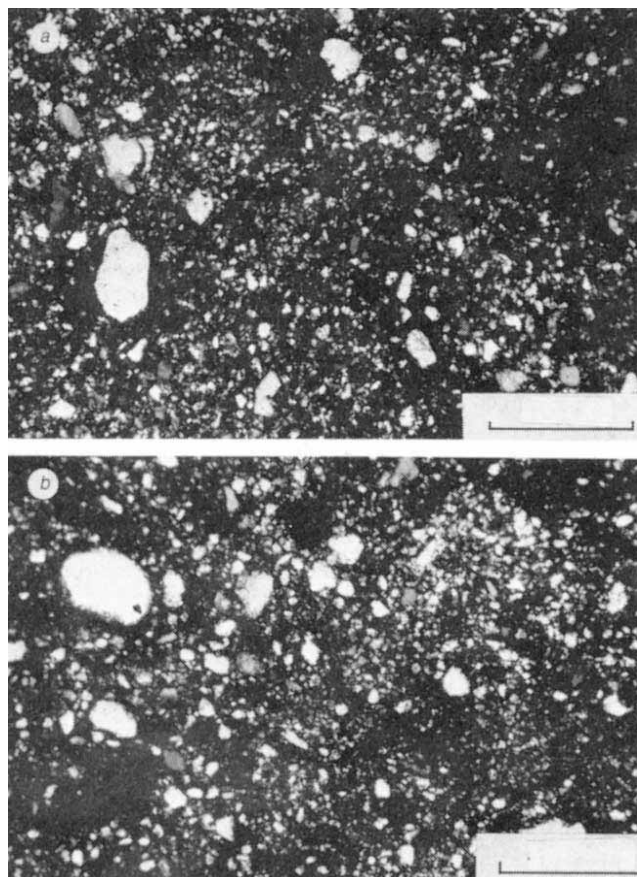


Fig. 2 Photomicrographs of *a*, F-fabric sarsen (sample C in Table 1) and *b*, F-fabric silcrete from Riversdale, South Africa. Both samples have a small skeletal grain fraction and titanium-rich matrix (2.08% TiO₂ in *a*, and 1.72% TiO₂ in *b*). Crossed polarisers. Scale bars, 500 μ m.

suggests several possible origins for sarsens. Probable local silica sources for N-WPS include direct dissolution of quartz grains and enhanced solubility quartz particles produced through grain abrasion, and release of silica during calcrete formation or from diatoms and other silica-secreting organisms. Silica may also be supplied from surface waters and atmospheric fall-out. A decrease in pH from >9 to <9, is considered to be a probable silica precipitating mechanism. Periodic changes in pH produced by dilution by fresh surface waters could produce alternating phases of high silica mobility (pH >9) and silica precipitation (pH <9). Alternatively, in the absence of pH changes, silica may gradually precipitate (particularly as overgrowths) from slowly migrating solutions only moderately supersaturated with respect to quartz. Water table fluctuations and downward percolation are regarded as the most viable models of N-WPS formation as these are the only mechanisms which allow the development of laterally extensive and relatively thick silcrete horizons.

Silica released within the weathering zone, either through bedrock weathering or clay mineral alteration (such as illite $\rightarrow$

kaolinite) provides the most likely silica source for WPS. Silica so released could become precipitated lower down the profile if drainage were sufficiently restricted. Aluminium mobilisation accompanying silica replacement of weathering profile clays would probably be associated with a low pH environment (pH <4) and titanium mobility and concentration in WPS supports this idea^{13,17}.

The proposed high pH environment and lack of associated deeply weathered sediments suggests a semi-arid or arid climate during N-WPS formation; a similar interpretation is appropriate for GS- and C-fabric sarsens. WPS probably formed under a relatively humid climate where rates of organic activity were sufficiently high to promote a low pH environment, and it is likely that titanium-rich F-fabric sarsens formed in similar conditions.

Future investigations of *in situ* sarsen equivalents in France (meulière) and elsewhere in mainland Europe associated with a more complete Cenozoic stratigraphic record should clarify the problem of dating sarsen formation and provide a basis for independent evidence for palaeoclimatic interpretation.

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Host specificity of temperate and tropical animals

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Many groups of animals show a gradient of increasing species richness from polar to equatorial regions^{1,2}. Theory^{3,4} suggests that species should be more specialised ecologically in the tropics. Most of the evidence for this comes from studies on birds^{5–10}. Birds appear to subdivide the habitat more finely and specialise more in their food in the tropics. It has been suggested¹¹ that tropical Papilionidae (Lepidoptera) are more specialised in their hosts than temperate species, but host specificity was considered only at the level of the plant family, and more detailed studies of a wide range of butterflies¹² suggest that they are little (if at all) more specialised in their food plants in the tropics. Similarly, monogenean trematode parasites of fish show no change in the degree of host specificity at different latitudes¹³. Here it is shown that one group of insects, the bark and ambrosia beetles (Coleoptera: Scolytidae and Platypodidae), are less host-specific in the tropics than in temperate regions, even though there are considerably more species present in the tropics. The 'reverse' latitudinal gradient in host specificity can be related to the greater proportion of relatively non-specific xylomycetophagous species (ambrosia beetles) in the tropics and to the greater heterogeneity of tropical forests which will make host specialisation more difficult.

Table 2 Relative frequency of micromorphological features and fabrics in sarsens, WPS and N-WPS from southern Africa

Micromorphological feature/fabric	Sarsens	N-WPS	WPS
Optically continuous overgrowths	A	U	X
Chalcedonic overgrowths	X	U	X
Glaebules (authigenic)	X	X	A
Colloform features	U	X	A
M-fabric	X	R	A
F-fabric	U	A	A
GS-fabric	A	U	X
C-fabric	C	U	X

A, abundant; C, common; U, uncommon; R, rare; X, absent. No. of samples: sarsens, 18; N-WPS, 29; WPS, 93.

Table 1 Comparisons of area, latitude and number of species of Scolytidae and Platypodidae in some temperate and tropical countries

Country	Area (km ²)	Latitudinal range	Total no. of species	Expected no. of species*
France	549,600	43–51°N	135	41.0–68.4
California	411,000	33–42°N	176	59.2–94.5
West Malaysia	131,300	1–6°N	512	256.9–345.2
Fiji	18,300	16–19°S	81	81

* No. of species expected in an area the size of Fiji, assuming a linear relationship between log (no. of species) and log (area) with a slope of 0.2–0.35.

Comparisons have been made between the bark and ambrosia beetle faunas of two temperate areas, France¹⁴ and California¹⁵, and two tropical areas, West Malaysia^{16,17} and Fiji¹⁸. The data obtained from refs 14 and 16–18 have been extended and/or updated by reference to many other sources. Table 1 indicates a gradient in species richness from the temperate to the tropical zone when area is taken into account. The islands of Fiji are perhaps no richer in species than California if equal areas are compared, but relatively fewer species are expected in isolated oceanic islands than on the mainland.

The data are classified according to host specificity and feeding habits of the beetles in Table 2. (Terms used to describe the feeding habits are defined in Table 2 legend.) Information on

Table 2 Number of species of Scolytidae and Platypodidae with different feeding habits confined to a particular species, genus or family of host plants or polyphagous

Country and feeding habits*	No. of species				
	Species	Genus	Family	Polyphagous	Total
France					
Phloeophagous	2	60	43	1	106
Herbiphagous	1	5	2	1	9
Spermatophagous	0	0	2	0	2
Xylophagous	0	0	0	1	1
Xylomycetophagous	0	2	3	9	14
Total	3	67	50	12	132
% Of fauna	2	51	38	9	100
California					
Phloeophagous	14	96	34	3	147
Herbiphagous	0	2	1	1	4
Spermatophagous	6	1	1	0	8
Xylophagous	0	1	1	1	3
Xylomycetophagous	0	1	5	5	11
Total	20	101	42	10	173
% Of fauna	12	58	24	6	100
West Malaysia					
Phloeophagous	0	3	21	8	32
Herbiphagous	0	5	3	10	18
Spermatophagous	0	1	1	12	14
Xylophagous	0	0	1	0	1
Xylomycetophagous	0	1	72	137	210
Total	0	10	98	167	275
% Of fauna	0	4	35	61	100
Fiji					
Phloeophagous	1	6	3	7	17
Herbiphagous	0	0	0	4	4
Spermatophagous	0	1	0	2	3
Xylophagous	0	0	0	0	0
Xylomycetophagous	0	1	6	25	32
Total	1	8	9	38	56
% Of fauna	2	14	16	68	100

* Phloeophagous species construct gallery systems and breed in the phloem and inner bark of woody plants; herbiphagous species in the stems of non-woody plants, in the pith of twigs or in leafstalks; spermatophagous species in seeds and fruits. Xylophagous species live and feed on the wood; xylomycetophagous species usually live in the wood but cultivate and feed on ambrosia fungi growing on the walls of their galleries^{19,20}.

both parameters is available for 98% of the species recorded in the temperate areas, but only 69% in Fiji and 54% in Malaysia. However, my own observations of the fauna of West Malaysia and of other tropical areas suggest that the main conclusions obtained here will not be significantly changed if further information becomes available for the tropical areas.

Errors are possible in the assignment of beetle species to different grades of host specificity. Thus if a host genus is monospecific in an area, or a host family is monogeneric, the true specificity of the beetle may be overestimated. Conversely, some species occasionally attack hosts outside their normal range. Breeding in such hosts may not occur, but the host may still appear in host lists. Errors in assignment are generally likely to overestimate host specificity and to be greater for the tropical areas. Corrections of such errors as more information becomes available will probably increase the observed differences between temperate and tropical areas.

Table 3 Values of χ^2 * based on percentages of species of Scolytidae and Platypodidae with different host specificity for pairs of countries

Species group	Countries compared†					
	Fr/Ca	Fr/Ma	Fr/Fi	Ca/Ma	Ca/Fi	Ma/Fi
All	10.5	81.3	74.2	107.4	88.0	15.9
Phloeophagous	10.9	63.9	54.5	91.3	46.0	52.5
Xylomycetophagous	13.0	15.9	8.7	12.8	22.7	7.6

* Values greater than 7.8 are significant at the 5% level; 11.3 at the 1% level.

† Ca, California; Fi, Fiji; Fr, France; Ma, West Malaysia.

The Platypodidae (all xylomycetophagous) have been lumped together with the Scolytidae because (1) they are probably no more than a divergent subfamily of Scolytidae²¹; and (2) the proportions of Platypodidae in the different grades of host specificity in West Malaysia are not significantly different ($\chi^2 = 3.1$) from the proportions found in the xylomycetophagous Scolytidae in that country. The family is only abundant in West Malaysia among the study areas (63 species included in Table 2). It forms a minor part of the fauna of the other areas (one species in California, two in France, five in Fiji).

The results of χ^2 tests carried out on the percentages of phloeophagous and xylomycetophagous species and all species together are shown in Table 3. Numbers of species in the groups with other feeding habits are too low for comparisons to be made. Comparing the total fauna of the four areas, it is clear that the tropical beetles as a whole are far less host specific than those of temperate areas. There is far greater similarity within the tropical or temperate zones than between them. Host specificity is greatest in California and least in Malaysia. One reason for this is apparent from the more detailed analysis. A far higher percentage of the fauna consists of xylomycetophagous species in the tropics. This group shows low host specificity (Table 2). Values of χ^2 are relatively low for all comparisons within this group of species, indicating a similar low specificity in both temperate and tropical regions. The ambrosia fungi, on which both adults and larvae feed, will grow in a great variety of trees. It must be remembered that the beetle, so far as is known, feeds on the same fungi whatever host it is attacking, and it is really the fungi that are polyphagous and not the beetle. However, it is the beetle that selects the host tree.

The host specificity of the phloeophagous species is similar in France and California, and higher than in the tropical countries. The difference is perhaps related to the greater species diversity and heterogeneity of tropical forests. Specialisation is only possible if the resource(s) on which specialisation occurs is renewed in a way that is reasonably regular relative to the dispersive ability and lifespan of the species concerned. Too great a scattering of the resource in space or time and the species can not specialise on it²². The beetle–host tree system has two

basic characteristics²³. The first is that the habitat stability²⁴ of the habitat unit (plant stem, leafstalk, fruit or seed) is approximately unity, so that only one beetle generation is possible in the unit. The second is that habitat units in a suitable state for colonisation form a spatial patchwork in the environment. Since each unit can be colonised for only a limited period, the spatial pattern continually shifts with time. As a result, the beetles are faced in each generation with the problems of relatively long-distance dispersal and host-finding. The problems are greater in tropical than temperate forests because of the higher species diversity and the more rapid processes of decay. In a highly heterogeneous environment, it can be advantageous to evolve strategies that reduce the apparent heterogeneity of the system from the point of view of the individual insect. One such strategy is to increase the degree of polyphagy²³.

The finding that host specificity of phloeophagous species is greater in Fiji than in West Malaysia is also contrary to current thought. Birds on islands tend to have broader habitats and eat more kinds of food than on the mainland^{8,25}. For the Fijian beetles, it is useful to distinguish between endemic and introduced species. The former are more host specific than the latter, and tend to be confined to the native forest trees¹⁸. The introduced species are mostly 'tramp' species which have been transported over wide areas of the tropics and are able to breed in both native and introduced trees. The greater host specificity of the endemic species may be related to the lower diversity of Fijian than Malaysian forests. From the point of view of the beetles, the Fijian forest is less heterogeneous and greater specialisation is possible. The introduced species with broader niches seem likely to gradually displace the endemic specialists^{26,27}.

The reasons for the low proportion of xylomycetophagous species in temperate areas, and of phloeophagous species in tropical areas are not clear²⁰. The ambrosia fungi presumably grow more slowly in colder climates, and the rate of production may be insufficient to keep the beetles and their offspring alive. The number of phloeophagous species in the tropics may be reduced by the greater abundance of Cerambycidae (longhorn beetles), many of which occur in the same niche, and whose larvae act as predators of bark beetle broods. Other factors are doubtless involved.

The data presented above do not support the usual view that species are more specialised in the tropics. Other animal taxa whose essential resources are more scattered in space and/or time in tropical than temperate latitudes may well show 'reverse' latitudinal gradients too. There is so little information available on the host specificity of invertebrates along latitudinal gradients that generalisations are premature.

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Swimming control in a cubomedusan jellyfish

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Although it has been established that swimming in scyphozoans is under control of rhopalial pacemakers and an associated subumbrellar nerve-net^{1–3}, little is known about this control at the cellular level. There is some doubt whether scyphozoan neurones have all-or-none action potentials^{3–6}. Here we show that subumbrellar neurones of the sea wasp *Carybdea rastonii* Haacke (Class Scyphozoa, Order Cubomedusae) propagate such action potentials, and that activity in these neurones originates in the rhopalial pacemakers and produces a contraction of the swimming musculature. The neurophysiology of swimming has not previously been studied in this order.

In order to determine the structure of the subumbrellar nerve-net of *Carybdea* we examined both live material and electron micrographs. Bipolar and tripolar neurones form an apparently random network throughout the subumbrella of *Carybdea*. The diameter of neurites ranges from 0.2 to 5 μm while the cell bodies can be up to 40 μm long. Interneuronal and neuromuscular synapses occur throughout the subumbrellar nerve-net.

Pacemaker activity is restricted to the rhopalia since jellyfish stopped swimming when we removed the rhopalia. When suction electrodes were attached to a rhopalium and to an adjacent region of the subumbrella, motor impulses originating in the rhopalium and conducted into the subumbrellar nerve-net were recorded. Potentials recorded over the nerve-ring during swimming consisted of two distinct components; a rapid (<10 ms) invariable biphasic spike, up to 80 μV , and a complex multiphasic spike of variable amplitude and duration (Fig. 1a). Changes in amplitude of the second component were associated with variations in the magnitude of muscular contractions (Fig. 1b). Therefore, we interpret the two components as an initial nerve spike followed by a compound muscle potential. As the interpulse interval of pacemaker spikes decreases, the amplitude of muscle potentials and contractions increases, suggesting neuroeffector facilitation. Excess Mg^{2+} (1:1 solution of

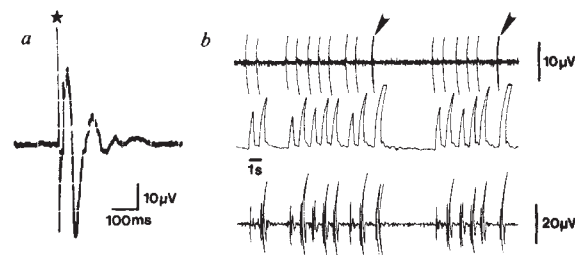


Fig. 1 a, An extracellular suction electrode covering the nerve-ring and surrounding subumbrellar muscle records a single, spontaneous swimming contraction. The rapid biphasic nerve spike (★) is followed by a complex muscle potential. b, Extracellular recordings from a rhopalial pacemaker site (top trace) and the nerve-ring of the opposite bell quadrant (middle trace). An uncalibrated force transducer record shows circular muscle contractions at a point midway between the two electrodes (bottom trace). The amplitude of the muscle contractions can be roughly correlated with the size of the compound muscle potentials of the bottom trace. Two double pacemaker spikes (arrows) give rise to double muscle potentials and a far larger contraction.

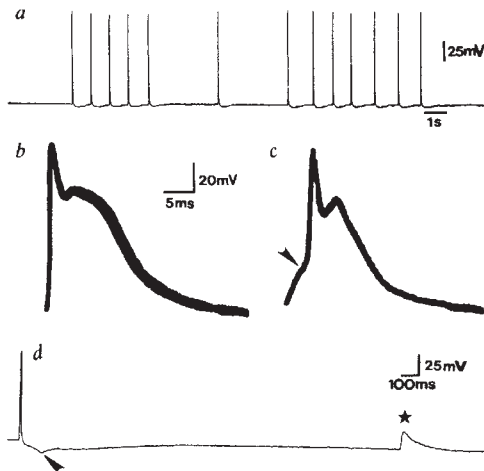


Fig. 2 *a*, Spontaneous intracellular action potentials from a subumbrellar motoneurone soma initiating 13 swimming contractions. Note the flat resting potential and the hyperpolarisations which follow each spike. *b*, Six superimposed action potentials from a motoneurone soma. In any one recording, there is little variation in the shape of the spike. *c*, The rapid depolarisation of a neuronal action potential was frequently superimposed on an apparent synaptic event (arrow). Similar synaptic events were occasionally recorded alone (*d*, ★). The notch seen in the spike after-hyperpolarisation (*d*, arrow) is a movement artefact caused by contraction of the surrounding muscles. d.c. amplification was used with 20–60 MΩ, 3 M KCl filled micropipette electrodes. The subumbrellar muscle sheet was dissected from one quadrant of the animal and pinned to a Sylgard base. A small circle of pins surrounded the penetrated neurone soma, which was visible with oblique substage illumination.

0.37 M MgCl₂: seawater) in the bath blocked muscular contractions within 5 min. This was followed by total cessation of all recordable electrical activity.

Cell bodies of bipolar and tripolar subumbrellar neurones gave stable recording for up to 15 min, when impaled with glass microelectrodes filled with 3 M KCl. Negative resting potentials of 72–76 mV were interrupted by all-or-none, positive-going action potentials of 105–120 mV and 12–20 ms duration (Fig. 2*a, b*). The slow membrane potential oscillations that have been recorded from the 'giant' neurones of hydromedusae^{7,8} were not seen. Action potentials consisted of a rapid rise to peak amplitude (1–2 ms risetime) and a slow partial decay with a second rounded peak (Fig. 2*b, c*). The action potential was followed by a 700–800 ms, 3–15 mV after-hyperpolarisation (Fig. 2*a*). In all but one record, in which a poor penetration was suspected, little variation in the shape of the action potential was evident during repetitive activity (Fig. 2*b*). In particular, the second peak did not vary with the strength of muscular contractions. The initial rise of many spikes was superimposed on a small 10–30 mV pre-potential (Fig. 2*c*). Occasionally similar depolarisations (200–300 ms duration) were recorded which did not trigger action potentials (Fig. 2*d*). We presume that these small amplitude events represent excitatory synaptic input from adjoining neurones of the net. Each action potential preceded a muscular contraction of the surrounding subumbrellar sheet, as judged by visual observation. Only occasionally were contractions not accompanied by spikes in the impaled neurone. In these instances, a conduction block was suspected, owing to the extensive pinning required to minimise movement near the electrode.

Those properties of the neuromuscular system of *Carybdea* which most clearly show it to be of the scyphozoan type are as follows. First, if the rhopalia, containing the pacemakers, are removed then there is no activity in the subumbrellar nerve-net and swimming ceases. Second, the flat resting potential (absence of pacemaker potentials) of subumbrellar neurones, together with the observation that action potentials in these cells seem to be driven by excitatory synaptic potentials, suggest that the

subumbrellar neurones are not autorhythmic but merely pass the motor pattern on to neighbouring cells of the net. These neurones function as both interneurones and motorneurones since spiking in subumbrellar neurones is associated with contractions of the underlying musculature.

Intracellular and extracellular electrophysiological recordings indicate that information in the subumbrellar nerve-net of *Carybdea*, and possibly other scyphozoans^{4,5}, is frequency coded with all-or-none impulses. The neuromuscular characteristics of swimming in *Carybdea*, described here, indicate that the Cubomedusae are closely related to other scyphozoans. This conclusion may be significant since the systematic position of cubomedusae has recently been questioned⁹.

All-or-none action potentials were recorded recently by Schwab in the subumbrellar neurones of another scyphozoan, *Cyanea* (personal communication).

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Fast and slow myosin within single skeletal muscle fibres of adult rabbits

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There is good evidence for the coexistence of different myosin types both in developing muscles^{1–3} and in Purkinje cells from adult chicken hearts⁴. In skeletal muscle fibres of adult animals, however, coexistence of fast (FM) and slow (SM) myosin has only been demonstrated after long-term electrical stimulation^{5,6}. The term 'promiscuity' has recently been coined⁷ to describe the coexistence of different myosin isoenzymes within a single fibre. Using novel, refined immunological methods we demonstrate here the presence of both FM and SM within single fibres of the musculus tibialis anterior of adult rabbits. Essentially identical results were also obtained with other muscles. Our findings imply that the genes coding for FM and SM can be expressed simultaneously within the same cell throughout an animal's entire life, and not only during development or after artificial electrical stimulation.

To test for the presence of FM and SM, antibodies of high specificity and high affinity⁸ were raised against rabbit FM, SM and the light chains of rabbit cardiac myosin^{9,10}. These antibodies were used in two types of assay. First, immunohistochemistry for myosin was performed on sections of shock-frozen muscle pieces and the reaction monitored by histochemical photometry. In addition, conventional histochemistry for myosin-ATPase¹¹, NADH-tetrazolium reductase¹² and phosphorylase¹³ allowed the fibres to be classified into fibre types^{11,14}. Combining the nomenclatures proposed by Brooke and Kaiser¹¹ and Peter *et al.*¹⁴, the fibres were designated as type I (slow-twitch-oxidative), type IIA (fast-twitch-oxidative-glycolytic), type IIB (fast-twitch-glycolytic) and type IIC (the very

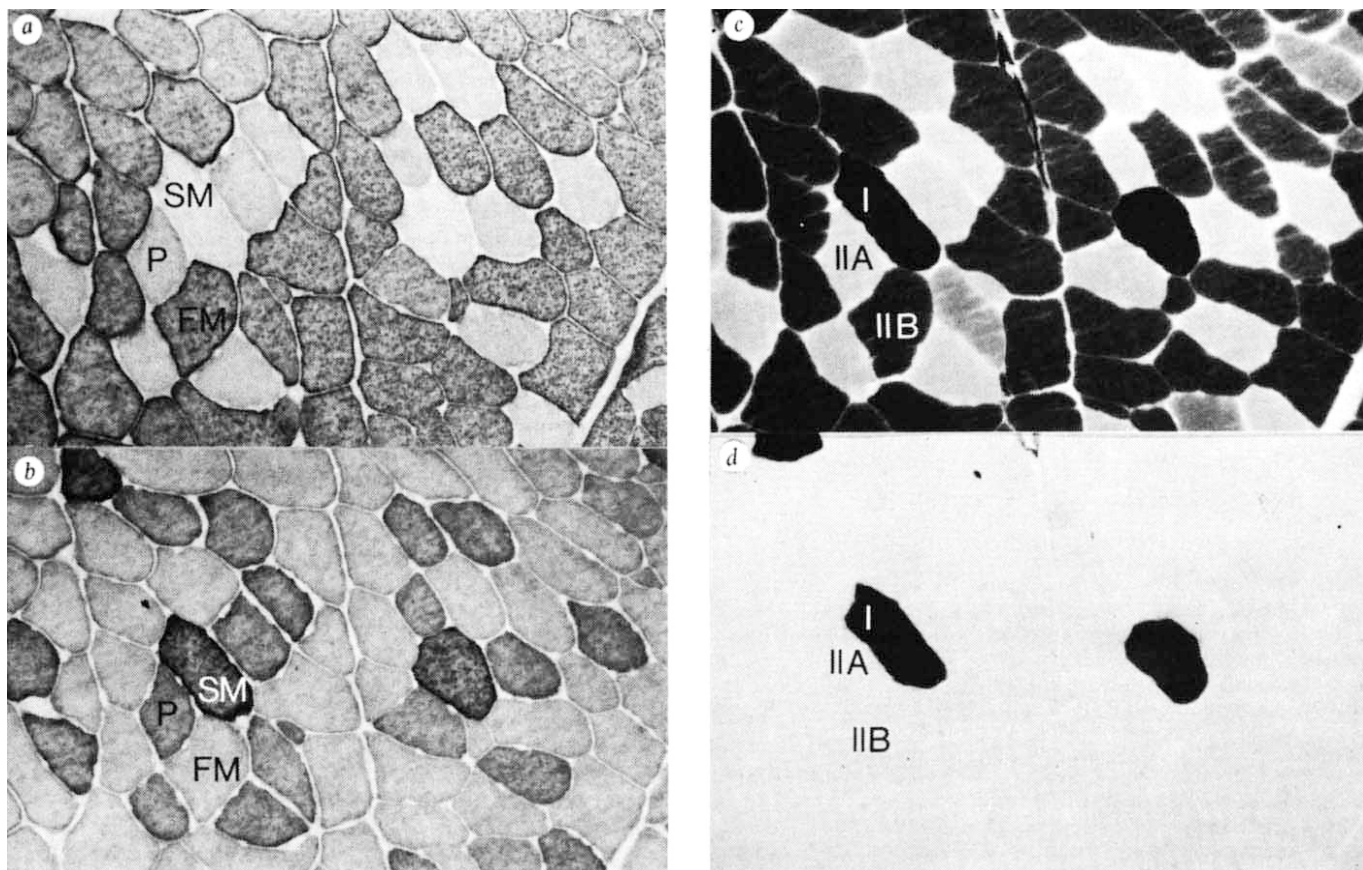


Fig. 1 Successive serial transverse sections of the musculus tibialis anterior of an adult (18 months old) rabbit analysed for FM (a), SM (b) and histochemical fibre type (c, d). Antibodies of high affinity and low heterogeneity were induced in high-responder guinea pigs using a long-term, low-dose immunisation programme⁸ and their specificities demonstrated as described⁹. Antibodies against the light chains of rabbit cardiac myosin were also prepared¹⁰ and analysed in the same way. Cryostat sections (8–10 μ m) of shock-frozen muscle pieces were reacted with antibody and the immuno-complexes visualised using a protein A-peroxidase technique optimised for our needs¹⁸. a, Reaction with anti-FM serum. b, Reaction with anti-SM serum; the same staining pattern was obtained with anti-cardiac-light-chain sera shown to cross-react strongly with the two light chains of SM but only negligibly with those of FM (Fig. 3a, b). c, d Staining for myosin-ATPase¹¹ after preincubation at pH 4.6 or 4.35, respectively, for 5 min as a means of classifying fibres into types I, IIA, IIB and IIC; identical results were obtained when staining was for NADH-tetrazolium reductase¹² and phosphorylase¹³ (data not shown). Note that some fibres, one of them marked P (promiscuous), react with both antisera (a, b); histochemically they belong to type IIA (c). $\times 150$.

rare type IIC fibres have been designated as undifferentiated fibres¹⁵). Second, myosin light chains from dissociated muscle tissue were analysed by combining classical gel electrophoresis¹⁶ with subsequent assay of the separated proteins by an enzyme-linked-immunosorbent-assay (ELISA) technique⁹.

When serial sections of the anterior tibial muscle were reacted with sera raised against FM (Fig. 1a) or SM (Fig. 1b) and the fibres histochemically typed (Fig. 1c, d), a clear correspondence of the staining patterns emerged. Type I fibres reacted with anti-SM sera only and type IIB fibres with anti-FM sera only. In contrast, type IIA fibres reacted with both antisera, as did the rarely occurring type IIC fibres. Close examination of the staining intensities of type IIA (P) fibres (Fig. 1, a–c) indicated the existence of subpopulations containing varying proportions of FM and SM. Computer-assisted analysis of the staining intensities of 132 randomly chosen fibres reacted with anti-FM and anti-SM sera revealed three well separated clusters (Fig. 2) corresponding to fibres containing only SM (7.3% of all fibres), only FM (61.4%) or both SM and FM in varying proportions (31.3%). Our results agree with those of Spamer and Pette¹⁷, who presented evidence suggesting the existence of subpopulations of type II fibres having a wide range of aerobic oxidative capacities and differences in their ability to oxidise fatty acids.

To confirm the presence of SM within type II fibres, 10 serial 40- μ m sections of the anterior tibial muscle were selected for absence of type I fibres. Following dissociation, the released proteins were separated by gel electrophoresis and analysed

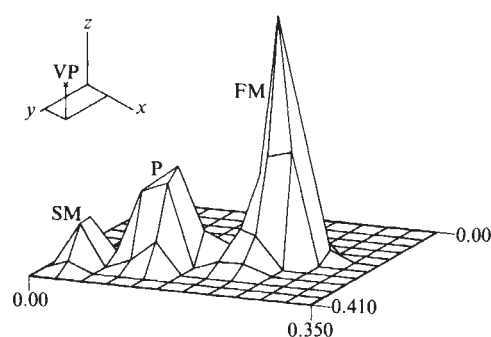


Fig. 2 Computer-assisted analysis of the FM and SM contents of 132 randomly chosen fibres from the anterior tibial muscle of an adult (18 months old) rabbit. Two successive transverse cryostat sections (8–10 μ m) were reacted with anti-FM or anti-SM, respectively, and developed using a protein A-peroxidase technique¹⁸. Protein A binds to the Fc portion of immunoglobulins (IgG) and the covalently attached peroxidase thus allows detection of antigenic sites. The absorbance of each fibre was measured (Leitz microscope photometer MPV 2), computed and plotted to give a relative frequency distribution. x, Absorbance after reaction with anti-FM serum. y, Absorbance after reaction with anti-SM serum. z, Relative frequency of absorbance values. VP, computer adjusted viewpoint for optimal three-dimensional impression. P, fibres containing both FM and SM; the width of this peak suggests the presence of subpopulations of fibres containing varying proportions of FM and SM.

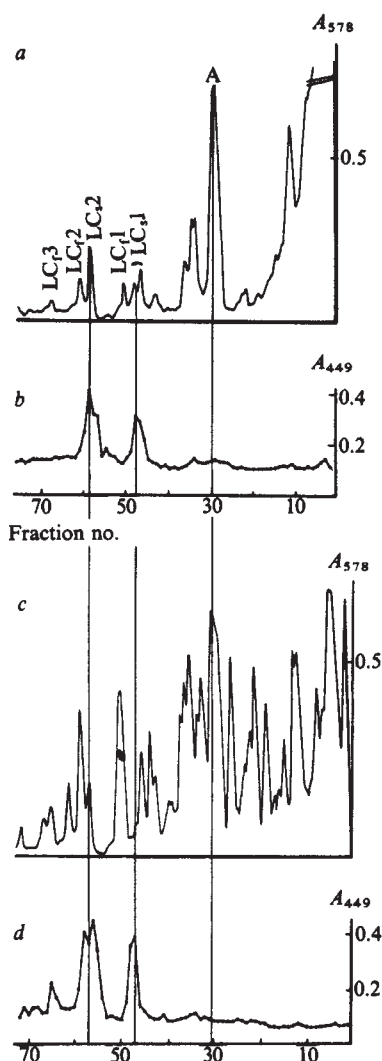


Fig. 3 Analysis of myosin light chains by a combination of 12% polyacrylamide gel electrophoresis and ELISA. *a*, Densitogram of gel stained with Coomassie brilliant blue after electrophoresis¹⁶ of an artificial test mixture obtained from dissociated (5 min at 98 °C in SDS sample buffer) myofibrils of fast and slow twitch fibres (~20 µg of protein each). *b*, Immunoreaction of the same proteins as in *a*: proteins were eluted from slices (1 mm segments) of a parallel gel, reacted with antiserum (dilution 1:100) raised against the light chains of rabbit cardiac myosin and assayed by an ELISA technique⁹; this antiserum reacted negligibly with light chains from FM alone but gave a trace identical to the one shown here with light chains from SM alone (not shown); anti-SM sera were not used in this assay as they only recognise LC₁ (ref. 9). *c*, Densitogram of gel stained with Coomassie brilliant blue after electrophoresis of total proteins from selected segments of anterior tibial muscle (adult rabbit, 18 months old); shock-frozen muscle pieces were serially sectioned (40 µm), the fibres typed histochemically in every fifth section, and 10 intervening sections chosen for absence of type I fibres; the sections were dissociated as in *a* and the proteins analysed by electrophoresis. *d*, Immunoreaction of the same proteins as in *c* separated on a parallel gel and analysed by an ELISA technique⁹ (serum dilution 1:75) as in *b*. Densitograms in *a* and *c* were aligned with respect to actin. A, actin; LC₁, LC₂ and LC₃, light chains of FM; LC₁ and LC₂, light chains of SM.

using an antiserum directed against the light chains of SM (Fig. 3*a, b*). This revealed two main peaks corresponding exactly to the positions of the two light chains of SM (Fig. 3*c, d*). This result supports the view that type II fibres also contain SM. However, identification of the SM-containing subtype(s), for example types IIA, IIB or IIC, was not possible.

There is already evidence that fibres contain both FM and SM. In rat diaphragm, Gauthier *et al.*¹ occasionally detected fibres

reacting with both anti-FM and anti-SM sera. In the anterior tibial muscle of rabbits we⁹ and Gröschel-Stewart¹⁹ found three populations of fibres, one of them giving an intermediate reaction with antibodies raised against either human pectoral muscle actomyosin or rabbit FM, respectively. In addition, reciprocal transformations of fibre types occurred in rats²⁰ and humans²¹ depending on physical activity, suggesting a profound change in the proportions of myosin isoenzymes. However, in histochemically identified single fibres of adult rabbit skeletal muscles, Weeds *et al.*²² and Pette and Schnez²³ could only demonstrate the presence of either FM or SM by microgel electrophoresis. Rubinstein *et al.*⁶ did not find fibres staining with anti-FM and anti-SM sera by direct immunofluorescence. These seemingly contradictory results can be explained if we consider the sensitivities of the methods used. We believe that a suitable sensitive immunological method can detect a smaller amount of one myosin species in a mixture of two than microgel electrophoresis of isolated single muscle fibres^{22,23}. The direct immunofluorescence technique⁶ has its well known limitations; we found that the more sensitive indirect immunofluorescence method was insufficient to detect unambiguously the presence of fibres containing both FM and SM⁹. From this we conclude that, in general, type IIA fibres probably contain very small amounts of SM.

An alternative explanation of our results could be the occurrence, in fibres reacting with both FM and SM antisera, of a hybrid myosin consisting of FM heavy chains and SM light chains. Such an hypothetical hybrid in adult muscles has been proposed by Hoh²⁴. Hybrids in developing muscles have been suggested by Rubinstein *et al.*² and by Pette *et al.*²⁵.

The results reported here demonstrate that the presence of FM and SM within single muscle fibres of adult animals occurs naturally. Weeds⁷ has recently pointed out that isoenzymes of myosin should provide useful markers for unravelling the early events in myogenesis and the differentiation of muscle fibres following innervation. In view of our recent observation that the myosin composition of muscle fibres changes with physical training (R.B., unpublished observation) and during ageing²⁶, we may add that the analysis of myosin isoenzyme composition could help in understanding the plasticity of muscle throughout life.

Since submission of this manuscript the presence of FM and SM within single fibres of adult rat soleus muscle has been demonstrated²⁷.

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'Square arrays' in the sarcolemma of human skeletal muscle fibres

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Fast twitch fibres of rat¹ and rabbit² show rectangular patterns of intramembrane particles in freeze-fracture preparations of the sarcolemma. These 'square arrays' are almost totally absent in the slow twitch soleus muscle of rat¹. I report here differences in the incidence of square arrays in human fetal and adult muscle and in different fibres within a human muscle. Square arrays probably classify fast and slow twitch fibres in freeze-fracture preparations of mixed muscles.

A fetal brachial biceps muscle was obtained from a male fetus weighing 385 g (20–24th week of gestation). Surgical biopsies were taken from the medial vastus muscle of two normal male subjects 29 and 36 yr old; the site of biopsy was close to the motor point. Specimens fixed in 2.5% glutaraldehyde were fractured at -100°C in a Balzers BAE 120 unit and replicated without etching.

Square arrays were found in all fibres from adult muscles but in none of the fetal muscle fibres. The arrays consisted of particles, 6–7 nm in diameter, attached to the P-face of the plasma membrane (Fig. 1); the E-face showed corresponding pits. In some fibres square arrays occurred in clusters and the individual arrays might contain up to 60 particles, whereas in other fibres arrays were dispersed randomly and rarely contained more than 20 particles. The numbers of square arrays per μm^2 plasma membrane determined in individual fibres formed a two-peak histogram; in fibres in which square arrays occurred frequently the individual arrays were larger than in fibres in which square arrays were rare (Fig. 2).

Thus, the frequency and size of square arrays of the plasma membrane of adult muscle fibres in man identify two classes of fibres; both are different from fetal muscle fibres. This was not due to the fact that junctional and non-junctional parts of the membrane were compared, because none of the preparations contained endplates. From the findings in fast and slow twitch muscles in rat¹ I conclude that the fibres with large square arrays that constitute the right peak of the histogram (Fig. 2) are fast twitch fibres and that those with smaller arrays that constitute

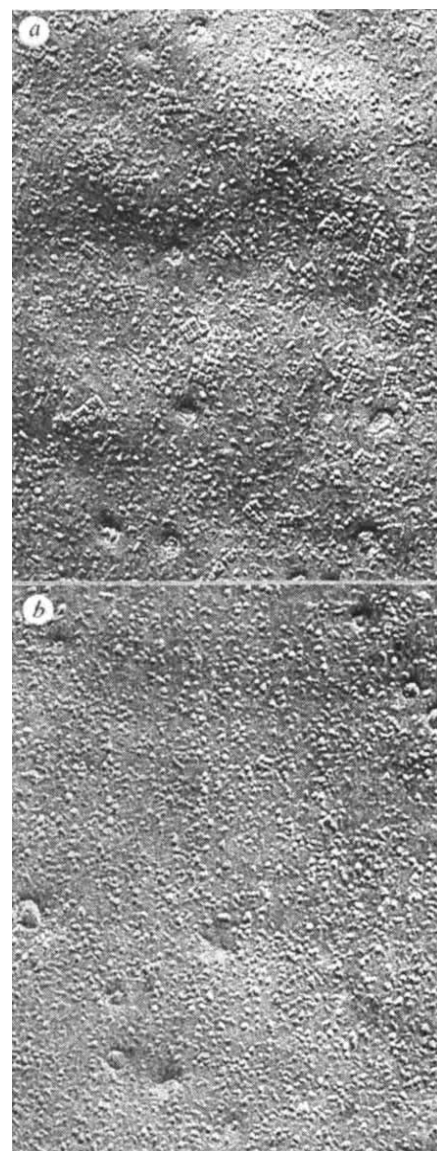


Fig. 1 Freeze-fractures of the medial vastus muscle of man. Both micrographs were taken from the same preparation, the direction of shadowing is from below. The P-faces of the sarcolemma of presumed fast twitch (a) and slow twitch (b) fibres are shown. Note the many orthogonal arrays of particles in a which are absent in b. Of each fibre, $22\ \mu\text{m}^2$ of the plasma membrane was studied; the number of square arrays was 11.1 per μm^2 and 1.3 per μm^2 in the fibres shown in a and b, respectively. Particles not contained in square arrays vary in diameter. The shallow invaginations represent openings of T-tubules or of caveolae of the plasma membrane. ($\times 72,000$).

Table 1 Quantitative findings in the P-face of the sarcolemma of human muscle fibres

	Adult muscle		Fetal muscle	Final magnification of micrographs
	Presumed fast twitch fibres	Presumed slow twitch fibres		
Square arrays per μm^2 mean (s.d.; n fibres)	5.9(3.07; 29)	1.3(0.85; 22)	—	$\times 60,000$
Intramembrane particles not in square arrays				
Particles per μm^2	949	909	929	$\times 400,000$
Size (nm) mean (s.d.; n particles)	9.7(1.9; 247)	9.4(1.3; 237)	10.5(2.4; 223)	$\times 400,000$
Caveolae per μm^2 mean (s.d.; n micrographs)	11.6(4.4; 40)	15.5(7.2; 32)	7.1(3.3; 10)	$\times 60,000$

In adult muscles, fibres were classified as presumably fast or slow twitch by size and arrangement of square arrays (unhatched and hatched areas in Fig. 2).

the left peak are slow twitch fibres. This difference is less obvious than in rat muscles, where fast twitch fibres display 5–15 square arrays per μm^2 and slow twitch fibres almost none¹. The fraction of fibres that were presumably fast (29 of 51, Table 1) agrees with the percentage of fibres that in frozen sections stained intensely for ATPase at pH 9.4 (52% and 57%, respectively). In normal mature muscle an intense reaction is thought to identify fast twitch fibres. In the fetal muscle studied all fibres reacted uniformly with quite high intensity.

Intramembrane particles attached to the cytoplasmic fracture face (P-face) but not contained in square arrays had the same diameter and were similar in number in fetal and adult and in fast and slow twitch fibres (Table 1). The differences in size were at most 1 nm and thus well below the resolution and reproducibility of the method. The number of this sort of intramembrane particles was higher (900 per μm^2) than in previous studies (250 per μm^2)^{3–5}. In these studies, particles were counted at low magnification ($\times 60,000$)^{4,5} and small particles may have been

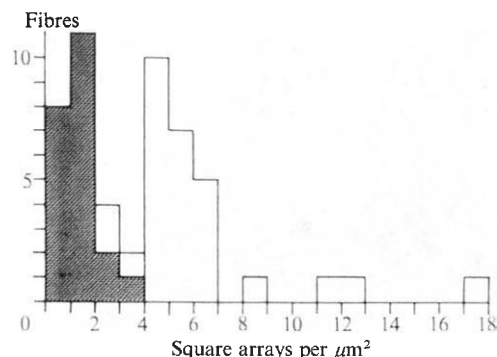


Fig. 2 Histogram of the frequencies of square arrays in 51 randomly selected fibres. Of each fibre, $22 \mu\text{m}^2$ of the P-face of the sarcolemma was investigated. The hatched area represents fibres with small and scattered square arrays, the unhatched area represents fibres with large and clustered square arrays.

overlooked. The size was not measured. Although it is possible that some intramembrane particles are artefacts due to condensation of water before shadowing, it is unlikely that the discrepancy is due to the fact that condensation of water was more pronounced in this than in the previous studies.

In rat soleus muscles cross-innervated by a nerve to a fast muscle, square arrays become more frequent; denervation alone does not change the number of square arrays⁶. As cross-innervation changes the contractile properties and the type of myosin⁷, this might suggest that innervation exerts a coordinated control of membrane and cytoplasmic aspects of muscle differentiation. Nevertheless, it is unlikely that the type of myosin and number of square arrays are determined by the same factor because fetal muscle fibres that lack square arrays contain a type of myosin that corresponds to myosin in fast twitch fibres of adult muscle⁸⁻¹².

There is evidence that intramembrane particles are proteins that, at least partly, determine the physiological properties of the plasma membrane; these properties differ in mature and immature and in fast and slow twitch fibres¹³⁻¹⁹. The number and size of particles not contained in square arrays were the same in the three sorts of fibres studied. This indicates either that square arrays, that is, the unknown protein constituting the particles within these arrays, determine specific properties of the membrane, or that structural differences of particles not contained in square arrays were too small to be demonstrated.

To speculate on the function of square arrays requires a comparison of findings in different species. Structures resembling square arrays are abundant in astrocyte membranes rich in $(\text{Na}^+ + \text{K}^+)\text{ATPase}$; hence, it has been suggested that the number of arrays is related to the activity of this enzyme in muscle fibres¹. The activity of $(\text{Na}^+ + \text{K}^+)\text{ATPase}$, however, is the same in plasma membranes of fast and slow twitch fibres²⁰. One could relate square arrays to a particular type of sodium channel, because only in mature, not immature, muscle fibres are action potentials blocked by tetrodotoxin¹⁸. Finally, one might consider the distribution of acetylcholine receptors, which is related strictly inversely to that of square arrays: in immature fibres, receptors are randomly distributed and square arrays are absent¹⁵, in fast twitch fibres, receptors are concentrated in the endplate region²¹ and square arrays occur everywhere except in the endplate region¹, and in slow twitch fibre some receptors also occur in the extra-junctional membrane¹⁹ where square arrays are less frequent than in fast twitch fibres.

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Use of chlorotetracycline fluorescence to demonstrate Ca^{2+} -induced release of Ca^{2+} from the sarcoplasmic reticulum of skinned cardiac cells

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It has been proposed that the trans-sarcolemmal influx of Ca^{2+} occurring during the plateau of the mammalian cardiac action potential is insufficient in itself to activate the myofilaments, but can trigger a release of Ca^{2+} from the sarcoplasmic reticulum (SR) which is sufficient for activation¹. The demonstration of this Ca^{2+} -induced release of Ca^{2+} relied entirely on experiments in which the tension developed by the myofilaments was used as a sensor of the changes of myoplasmic free Ca^{2+} concentration ($[\text{free Ca}^{2+}]$) in segments of single cardiac cells from which the sarcolemma had been removed by microdissection (skinned cardiac cells)¹. The small size of these preparations has previously prevented the use of more direct methods for the detection of myoplasmic Ca^{2+} movements²⁻⁴. The present study is a direct demonstration of Ca^{2+} -induced release of Ca^{2+} from the SR of skinned cardiac cells treated with chlorotetracycline (CTC), a fluorescent chelate probe which enables changes in the amount of Ca^{2+} bound to a variety of biological membranes or micelles to be monitored⁵⁻⁸. The fluorescence increases when more Ca^{2+} is bound.

Skinned cardiac cells, $10 \mu\text{m}$ wide and $50 \mu\text{m}$ long, from the rat ventricle were prepared as described previously¹. It was essential to limit the duration and intensity of illumination used for fluorescence excitation so as to avoid a progressive deterioration of the Ca^{2+} accumulation in and release from the SR. Microdissection and attachment of the skinned cell to the microtools were carried out using the regular low-voltage transmitted light of the Biovert inverted microscope (Reichert). Then, in the absence of any illumination, the skinned cell was superfused for 10 min with $5.0 \times 10^{-5} \text{ M}$ CTC in a solution containing $3.2 \times 10^{-8} \text{ M}$ free Ca^{2+} and $3.2 \times 10^{-4} \text{ M}$ free Mg^{2+} , at pH 7.10 and 22°C . To eliminate any contractile movement during Ca^{2+} release from the SR which would have disturbed the optical recording, a high concentration ($1.0 \times 10^{-3} \text{ M}$) of EGTA was used in all the solutions. The $[\text{free Ca}^{2+}]$ of $3.2 \times 10^{-8} \text{ M}$ has been shown to result in only slightly less than optimal Ca^{2+} loading of the SR⁹. The cell was then washed for 5.0 s in the same Ca^{2+} -containing solution but in the absence of CTC. Only at this time was the shutter open; the fluorescence was excited with the incident illumination of the Biovert microscope through an interference filter (with peak transmission at 400 nm and a bandwidth of 4 nm at half height), a heat absorption filter and an exciter filter. The fluorescence emission was collected with a

×60 objective (0.90 n.a.) through a monochromator adjusted at 530 nm, and was measured with a photomultiplier as part of a modified high-sensitivity Reichert microspectrophotometer.

When the perfusion containing 3.2×10^{-8} M free Ca^{2+} was replaced by one with 1.8×10^{-7} M free Ca^{2+} , there was a rapid decrease in fluorescence (Fig. 1a). Perfusion with 1.0×10^{-8} M free Ca^{2+} caused a slight increase in fluorescence, and 3.2×10^{-8} M free Ca^{2+} a larger fluorescence. In contrast, a further

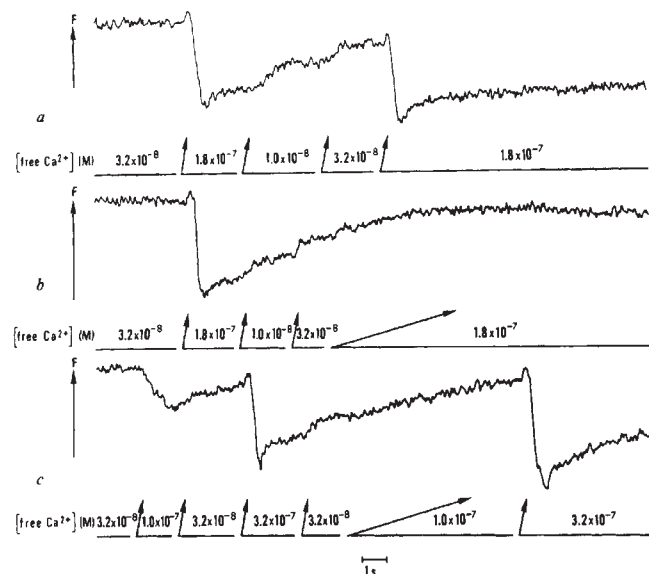


Fig. 1 Effects of varying the myoplasmic [free Ca^{2+}] in skinned cardiac cells on the fluorescence of chlorotetracycline (US Biochemical Corporation). The arrow labelled F at the left of each tracing indicates an increase in fluorescence and the size of the arrow corresponds to 5% of the fluorescence measured during the initial perfusion with 3.2×10^{-8} M free Ca^{2+} (measurement done during a 1.0-s opening of the shutter, not shown). The arrows below each tracing indicate the time and rate of the solution changes. A slow variation of the [free Ca^{2+}] between two values was obtained by varying the relative output of two simultaneous perfusions with two solutions of [free Ca^{2+}] equal to the initial and terminal values, respectively. The perfusion solutions contained 1.0×10^{-3} M total EGTA, 3.2×10^{-3} M Mg-ATP, 3.2×10^{-4} M free Mg^{2+} , 1.5×10^{-2} M creatine phosphate, 15 U ml $^{-1}$ creatine phosphokinase, 0.160 M ionic strength with K^{+} and Cl^{-} as major ions, and 1.0×10^{-1} M sucrose. The pH was maintained at 7.10 with 3.0×10^{-2} M *N*-tris(hydroxymethyl)methyl-2-aminoethanesulphonic acid (TES), which is sufficient for pH buffering during Ca^{2+} release from the SR of skinned cardiac cells⁹. The stability constants and the computer program used in the calculation of the multiple equilibria between metals and ligands have been reported in detail elsewhere⁹. In particular, the stability constant described by Allen and Blinks was used for the Ca-EGTA complex (see ref. 9 for refs and correspondence to other stability constants). An apparent stability constant of 7.53×10^2 M $^{-1}$ from the Ca-CTC complex was included in the computer program although this binding is much smaller than the binding of EGTA to Ca. Experiments similar to that of a except that the 5.0 s washing and the increase in [free Ca^{2+}] from 3.2×10^{-8} M to 1.8×10^{-7} M were applied at various intervals after the beginning of the CTC-containing perfusion (and experiments using caffeine for inducing a fluorescence decrease) demonstrated that the mean value of the half-time of the CTC perfusion necessary to obtain a maximum amplitude of transient decrease of fluorescence was 176 s. During a continuous perfusion with 3.2×10^{-8} M free Ca^{2+} in the absence of the CTC, there was a progressive decrease in the amplitude of the transient fluorescence decrease. The half-time for this decrease was 142 s when the preparation was illuminated by opening the shutter only at the time of the increase of the [free Ca^{2+}] to 1.8×10^{-7} M. The consistency between the half-times for the increase (176 s) and decrease (142 s) in the amplitude of the fluorescence transient suggests that the decrease was mainly caused by a progressive leakage of CTC from the SR. Additional mechanisms such as a weak Ca^{2+} -ionophoretic effect of CTC¹⁷ could have a minor role. However, during continuous illumination the amplitude of the fluorescence transient decreased much faster because of photodynamic damage to the Ca^{2+} accumulation in and release from the SR. Accordingly, conclusions were derived only from experiments in which, as in c, the amplitude of the transient was larger during the second triggering than during the first one. The findings would have been even more dramatic if corrections for the progressive deterioration had been made. Finally, the simplest explanation for the small and transient increase in fluorescence that preceded the Ca^{2+} -induced decrease is to attribute it to the effect of reaching myoplasmic [free Ca^{2+}] values intermediary between 3.2×10^{-8} M and 1.8×10^{-7} M during the time required for perfusion change (0.2 s). This would explain the finding that the fluorescence increased further until a [free Ca^{2+}] sufficient to trigger Ca^{2+} release had been reached.

increase in [free Ca^{2+}] to 1.8×10^{-7} M caused an abrupt decrease in fluorescence.

Although CTC binds to Mg^{2+} as well as to Ca^{2+} , the wavelength of 400 nm selected for excitation favoured the fluorescence of the Ca^{2+} chelate over that of the Mg^{2+} chelate. When excitation was at wavelength 375 nm, which favours the Mg^{2+} chelate, the same increase in [free Ca^{2+}] from 3.2×10^{-8} M to 1.8×10^{-7} M produced a decrease in fluorescence of less than 1% of the maximum fluorescence (defined in Fig. 1 legend). In contrast, a decrease of [free Mg^{2+}] from 3.2×10^{-4} M to 3.2×10^{-5} M, in the presence of a constant [free Ca^{2+}] at 3.2×10^{-8} M, produced a fluorescence decrease of 3% and 14% at excitation wavelengths of 400 and 375 nm respectively. Thus, the changes of fluorescence shown in Fig. 1a directly monitored changes in the amount of bound Ca^{2+} , rather than changes in the amount of bound Mg^{2+} resulting from the Ca^{2+} translocation.

The changes in fluorescence intensity (Fig. 1a) did not correspond to changes in the amount of Ca^{2+} bound to the mitochondria because results similar to those shown in Fig. 1a were obtained in skinned cardiac cells treated with 5.0×10^{-3} M azide or 5.0×10^{-6} M carbonyl cyanide *p*-trifluoromethoxyphenylhydrazide, which should inhibit the energy-linked Ca^{2+} transport by the mitochondria¹⁰, or in cells treated with 1.0×10^{-6} M ruthenium red, which should inhibit passive Ca^{2+} binding to the mitochondria¹⁰. These findings are consistent with previous results from tension recordings in skinned cardiac cells suggesting that the mitochondria do not interfere with either the active transport or the passive binding of Ca^{2+} when the myoplasmic [free Ca^{2+}] is lower than 1.0×10^{-6} M (refs 1, 9). Furthermore, the classical data supporting the evidence that isolated mitochondria present high-affinity binding sites for Ca^{2+} effective at concentrations lower than 1.0×10^{-6} M have recently been claimed to be artefactual^{11,12}. A rapid decrease in fluorescence similar to that shown in Fig. 1a could be induced by 1.0×10^{-2} M caffeine or 1.0×10^{-5} M of the ionophore A23187. Both interventions release Ca^{2+} from the SR⁹. The amplitude of the fluorescence decrease induced by increasing the [free Ca^{2+}] was about half of that induced by caffeine and one-quarter of that induced by A23187. In contrast, no decrease in fluorescence was induced by either increasing the [free Ca^{2+}] or adding caffeine or ionophore in cells treated with 0.5% of the detergent Brij 58, which destroys the ability of the SR actively to accumulate and release Ca^{2+} (refs 1, 9). Accordingly, the transient changes in fluorescence intensity (Fig. 1a) were entirely attributed to changes in the amount of Ca^{2+} bound by the SR. Note, however, that the preparation retained about 75% of its maximum fluorescence after treatment with Brij 58 and that about 20% of the fluorescence was background fluorescence already present before the application of CTC.

Using a Millipore filtration technique for monitoring changes in the CTC content of the medium bathing SR vesicles, Millman and Haynes¹³ have recently demonstrated that CTC penetrates the SR. They concluded that both the influx and efflux of CTC are much slower than those of Ca^{2+} . These findings are consistent with the results obtained from skinned cardiac cells and summarised in Fig. 1 legend. Thus, the 10-min perfusion that preceded the tracing shown in Fig. 1a permitted the slow diffusion of CTC into the SR and its complexation with the Ca^{2+} bound to the SR membrane. As the [free Ca^{2+}] inside the SR attains a rapid equilibrium with the Ca^{2+} bound to the SR membrane, a decrease in the [free Ca^{2+}] inside the SR caused by Ca^{2+} release should decrease the amount of Ca^{2+} bound, and this should result in a decrease in fluorescence intensity⁵⁻⁸. Accordingly, the rapid fluorescence decreases observed in Fig. 1a are interpreted as a demonstration of Ca^{2+} release. Because of the slow efflux of CTC, most of the CTC that had penetrated the SR during the 10-min loading period remained trapped there after the Ca^{2+} release which caused the initial decrease of fluorescence (Fig. 1a). The subsequent application of solutions with increasing [free Ca^{2+}] caused a reloading of the SR with Ca^{2+} which resulted in a recovery of the fluorescence. Conversely, the sudden decrease in fluorescence observed when the

[free Ca^{2+}] of the solution reached 1.8×10^{-7} M demonstrates a Ca^{2+} -induced release of Ca^{2+} from the SR.

The minimum [free Ca^{2+}] required for triggering a Ca^{2+} -induced fluorescence decrease varied between 5.6×10^{-8} M and 1.8×10^{-7} M in 22 experiments of the type shown in Fig. 1a. These values are within the same range as the [free Ca^{2+}] required for Ca^{2+} -induced release of Ca^{2+} from the SR previously inferred from tension recordings in skinned cardiac cells⁹, and are lower than the [free Ca^{2+}] necessary to induce a detectable tension (about 1% of maximum) by the direct activation of the myofilaments, which was between 3.2×10^{-7} M and 5.6×10^{-7} M. Finally, the rate of the Ca^{2+} -induced decrease of fluorescence is in the same range as the rate of tension development in the intact rat ventricle at 22 °C.

All the experiments shown in Fig. 1 were carried out in the presence of 3.2×10^{-4} M free Mg^{2+} to permit comparison with our previous experiments on tension recordings that were done at this [free Mg^{2+}] (refs 1, 9). It has recently been suggested that the intracellular [free Mg^{2+}] of the intact muscle cell may be 10 times higher¹⁴. However, in eight experiments similar to that of Fig. 1a, but carried out in the presence of 3.2×10^{-3} M free Mg^{2+} , the [free Ca^{2+}] required to trigger a Ca^{2+} -induced decrease of fluorescence ranged between 1.0×10^{-7} M and 3.2×10^{-7} M. This was still less than the [free Ca^{2+}] required to induce a detectable tension by the direct activation of the myofilaments, which was 5.6×10^{-7} – 1.0×10^{-6} M at this [free Mg^{2+}].

The preceding results (Fig. 1a) demonstrate directly a Ca^{2+} -induced release of Ca^{2+} from the mammalian cardiac SR. Fig. 1b and c correspond to additional findings which may help in understanding the mechanism of this process and in evaluating its relevance to the excitation-contraction coupling of the intact cardiac muscle. One of these findings is that the Ca^{2+} -induced release of Ca^{2+} depends not only on a critical level of [free Ca^{2+}] being reached in the myoplasm but also on the rate at which this change of myoplasmic [free Ca^{2+}] takes place. The initial part of Fig. 1b shows that increasing the [free Ca^{2+}] from 3.2×10^{-8} M to 1.8×10^{-7} M within 0.2 s caused a Ca^{2+} -induced decrease of fluorescence in this cell similar to that observed in the experiment shown in Fig. 1a. The cell was then subjected to the same succession of perfusions as in the experiment of Fig. 1a except that the second increase in [free Ca^{2+}] from 3.2×10^{-8} M to 1.8×10^{-7} M was effected very slowly, in 5.0 s. This resulted in no Ca^{2+} release but in a continued increase of fluorescence corresponding to further loading of the SR. With such a slow (5.0 s) perfusion change a minimum [free Ca^{2+}] in the range of 3.2×10^{-7} – 5.6×10^{-7} M (17 experiments) was necessary to induce a Ca^{2+} release in the presence of 3.2×10^{-4} M free Mg^{2+} .

Although the mechanism of Ca^{2+} -induced release of Ca^{2+} is unknown, it was generally believed that the trigger was simply an increase of [free Ca^{2+}] outside the SR^{1,2} or the gradient of [free Ca^{2+}] across the SR membrane⁹. The present results (Fig. 1b) indicate that this is not the case, although more experiments will be needed to define the exact relationship between the two variables—the level of [free Ca^{2+}] and the rate of change of [free Ca^{2+}]—on which the Ca^{2+} release depends. With respect to the physiological role of the Ca^{2+} -induced release of Ca^{2+} from the SR, the present findings (Fig. 1b) may help to explain some aspects of the force-frequency relationships of the intact heart. For example, it may be proposed that for each beat a rapid initial Ca^{2+} influx occurring during the plateau of the action potential may trigger the release of Ca^{2+} stored in the SR. This may be followed by a slower influx of Ca^{2+} , loading the SR with an amount of Ca^{2+} which will be available for release during subsequent beats.

Another finding is that the magnitude of Ca^{2+} -induced release of Ca^{2+} can be increased by at least two mechanisms: (1) an increase in the level of the [free Ca^{2+}] used as a trigger, and (2) an increase in the level of Ca^{2+} loading of the SR just before the triggering of the Ca^{2+} release. The initial part of the tracing of Fig. 1c shows that 3.2×10^{-7} M free Ca^{2+} induced a larger and much faster release of Ca^{2+} than was induced by 1.0×10^{-7} M

free Ca^{2+} , even though the rate of perfusion change and, presumably, the level of Ca^{2+} loading were unchanged.

The end of the tracing of Fig. 1c shows that a slow increase in [free Ca^{2+}] from 3.2×10^{-8} M to 1.0×10^{-7} M did not induce a Ca^{2+} release (in contrast to the effect of a fast increase to the same level seen at the beginning of the tracing), but produced a continued increase in fluorescence. Note that the fluorescence remained below the level observed during the initial perfusion with 3.2×10^{-8} M free Ca^{2+} because the CTC content of the SR was the limiting factor and had decreased slowly during the experiment. However, the larger loading of the SR is unequivocally demonstrated (see discussion in Fig. 1 legend) by the greater magnitude of the fluorescence decrease induced by the rapid increase in [free Ca^{2+}] to 3.2×10^{-7} M after its slow increase to 1.0×10^{-7} M.

This gradation of the Ca^{2+} release demonstrated in Fig. 1c is relevant to the understanding of the mechanism of the Ca^{2+} -induced process, which must be more complex than the all-or-none process originally proposed¹⁵. It is also relevant to the compatibility of this process with the known gradation of cardiac contraction with the extracellular [Ca^{2+}] and with the magnitude of the trans-sarcolemmal Ca^{2+} current¹⁶.

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Specific tricyclic antidepressant binding sites in rat brain

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The discovery of high-affinity binding sites for psychoactive drugs such as benzodiazepines^{1,2}, opiates³ and neuroleptics⁴ has opened up new approaches to the study of these drugs and their mechanisms of action. Although most tricyclic antidepressants inhibit neuronal uptake of noradrenaline and serotonin⁵, their mechanism of action remains unclear. Changes in the sensitivity of the β -receptor after chronic tricyclic antidepressant treatment^{6,7} suggest that they modulate noradrenergic neurotransmission. Tricyclic antidepressants also act directly on cholinergic⁸, histaminergic⁹, α -adrenergic¹⁰ and serotonergic¹¹ receptors. It is not clear, however, which, if any, of these effects

are related to the primary antidepressant effect or whether they are simply responsible for some of the side effects. We have thus investigated the possibility that specific binding sites for tricyclic antidepressants exist in the central nervous system. So far, binding studies using ^3H -labelled tricyclic antidepressant drugs have only detected binding to histaminergic H_2 and cholinergic muscarinic receptors¹² and low-affinity binding¹³. We demonstrate here a population of specific high-affinity binding sites for ^3H -imipramine on brain membranes which may be responsible for the antidepressant effects of these drugs.

Cerebral cortex dissected from 180–250 g male Sprague-Dawley rats was homogenised in 50 volumes of buffer (50 mM Tris-HCl, pH 7.4, 120 mM NaCl, 5 mM KCl) and centrifuged at 30,000g for 10 min. The pellet was twice resuspended in the same buffer and centrifuged. The final, washed pellet was resuspended in 33.3 volumes of buffer (final protein concentration $\sim 3 \text{ mg ml}^{-1}$). The binding of ^3H -imipramine was measured at 0 °C after incubating 180 μl of membrane suspension with ^3H -imipramine (0.5–20 nM) (specific activity 29.8 Ci mmol^{-1} , NEN) in a total volume of 250 μl for 60 min. 100 μl was taken and rapidly diluted into 5 ml ice-cold buffer and immediately filtered through Whatman GF/F glassfibre filters. The filters were washed with $3 \times 5 \text{ ml}$ ice-cold buffer, dried and the radioactivity measured by liquid scintillation spectrometry. The specific binding of ^3H -imipramine was defined as the difference between the total binding and that remaining in the presence of 10 μM desipramine. At 5 nM ^3H -imipramine, specific binding was 60% of the total binding.

Specific high-affinity ^3H -imipramine binding was saturable (Fig. 1) whereas the nonspecific binding increased linearly with ^3H -imipramine concentration. A Scatchard analysis of the specific binding (Fig. 1b) gave a straight line, indicating a single population of non-interacting sites. The mean apparent dissociation affinity constant, K_d , was $4.0 \pm 0.5 \text{ nM}$ (mean $\pm$ s.e.m., $n = 13$) and the total number of binding sites, B_{max} , was $13.8 \pm 1.3 \text{ pmol per g original wet tissue weight}$. Hill analysis (not shown) gave a Hill number of 1.04, indicating a lack of cooperativity. ^3H -imipramine binding reached equilibrium rapidly even at 0 °C (Fig. 2). The $t_{1/2}$ for association at 2.5 nM ^3H -imipramine was 5 min. Dissociation, induced by an excess (10 μM) of

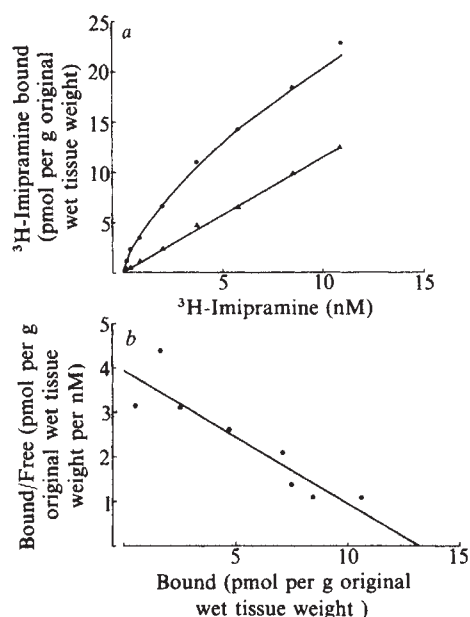


Fig. 1 Binding of ^3H -imipramine to rat cortical membranes. ^3H -imipramine at 0.1–12 nM was incubated in duplicate with or without excess desipramine (10 μM). a, Total binding (●) and that not displaced by 10 μM desipramine (▲) (nonspecific binding). b, The specific binding as a Scatchard plot ('bound', specifically bound ^3H -imipramine (pmol per g original wet tissue weight); 'free', free ^3H -imipramine (nM)).

Table 1 Inhibition of ^3H -imipramine binding in rat cortex

	IC ₅₀ (nM)		IC ₅₀ (nM)
Tricyclic anti-depressants		Monoamine uptake blockers	
Imipramine	7	Fluoxetine	200
Desipramine	18	Nisoxetine	200
Protriptyline	20	Quipazine	700
Chloripramine	25	Cocaine	900
Amitriptyline	25		
Doxepin	300	Other drugs	
Atypical anti-depressants		Phentolamine	200
Iprindole	5,500	Chlorpromazine	200
Viloxazine	11,500	Pyrilamine	500
Mianserin	20,000	Serotonin	1,000
		WB 4101	2,000
		Atropine	10,000
		Histamine	20,000

IC₅₀ is the concentration required to inhibit 50% of the specific ^3H -imipramine binding at 5 nM. The following compounds were inactive (IC₅₀ greater than 100 μM): pargyline, noradrenaline, dopamine, yohimbine, clonidine, methysergide, carbamylcholine, spiroperidol, cimetidine, Met-enkephalin, substance P, amphetamine.

desipramine, was also rapid, complete displacement being achieved within 60 min ($t_{1/2}$, 5 min).

The distribution of ^3H -imipramine binding sites varied among the major brain regions (cortex, striatum, cerebellum, hypothalamus). The greatest binding was found in the hypothalamus (K_d , $5.21 \pm 3.4 \text{ nM}$; B_{max} , $15.97 \pm 2.71 \text{ pmol per g original wet tissue weight}$, $n = 4$), which had seven to eight times more receptors than the region of lowest binding, the cerebellum (K_d , $8.0 \pm 3.1 \text{ nM}$; B_{max} , $2.20 \pm 0.39 \text{ pmol per g original wet tissue weight}$, $n = 3$). No ^3H -imipramine binding was detected in the heart.

All the tricyclic antidepressants studied are normally used clinically in the range of 60–300 mg per day. Five of these displaced ^3H -imipramine binding with very similar affinities (Table 1). Doxepin, however, was about 10-fold less potent. This difference may reflect a different absorption, distribution and metabolism for this drug *in vivo*. Tricyclic antidepressants, atypical antidepressants and monoamine uptake-blocking agents inhibited ^3H -imipramine binding (Table 1). However, the binding site for ^3H -imipramine seems to be distinct from the neuronal noradrenaline uptake site; cocaine, which blocks noradrenaline uptake, inhibits ^3H -imipramine binding fairly actively, but amphetamine, which *in vitro* is almost equipotent with cocaine in inhibiting noradrenaline uptake, is completely inactive at displacing the binding of ^3H -imipramine. Furthermore, ^3H -imipramine binding sites are absent in the rat heart whereas noradrenaline uptake is very active in this organ. Finally, a comparison of the inhibition of the uptake of dopamine, noradrenaline and serotonin by antidepressants with their potencies for the inhibition of ^3H -imipramine binding

Table 2 ^3H -Imipramine binding and monoamine uptake inhibition

	^3H -imipramine binding (IC ₅₀ , μM)	Uptake inhibition (IC ₅₀ , μM)		
		DA	NA	5-HT
Imipramine	0.007	12.5	0.02	0.24
Desipramine	0.018	8.7	0.0015	2.24
Protriptyline	0.020	4.9	0.003	1.62
Chloripramine	0.025	3.8	0.044	0.015
Amitriptyline	0.025	5.6	0.020	—
Iprindole	5.5	7.9	2.6	10.0
Mianserin	20.0	19.0	0.084	11.0

The IC₅₀ values for ^3H -imipramine binding are taken from Table 1. The uptake inhibition data are taken from Randrup and Braestrup¹⁷. DA, dopamine; NA, noradrenaline; 5-HT, Serotonin.

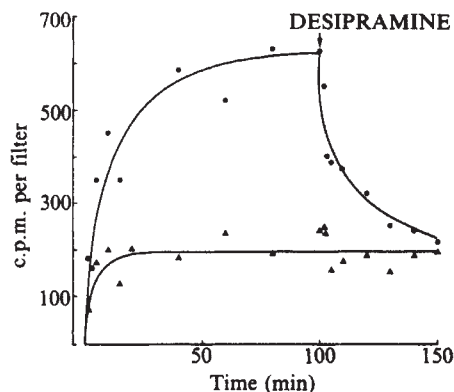


Fig. 2 Time course of ^3H -imipramine total (●) and nonspecific (▲) binding. 2.5 nM ^3H -imipramine was incubated with the membrane suspension at 0°C as described in the text and samples were filtered at various times. At 100 min desipramine (10 μM) was added to displace the specifically bound ^3H -imipramine.

(Table 2) shows them to be unrelated. Desipramine, for example, which is at least 10 times more active than imipramine at inhibiting noradrenaline uptake and 10 times less active at inhibiting the uptake of serotonin, is almost equiactive with imipramine at inhibiting ^3H -imipramine binding. Although various other compounds were weakly active in inhibiting specific ^3H -imipramine binding (Table 1), there is no clear pattern or obvious relationship with other known pharmacological activities. Muscarinic and histaminergic H_2 receptors, which have been implicated in the action of tricyclic antidepressants,^{8,9} do not seem to be involved, as atropine and cimetidine (muscarinic and H_2 histaminergic antagonists, respectively) are inactive.

It is difficult to establish a correlation between an *in vitro* biochemical activity of a drug and its clinical therapeutic efficacy in depression because of the heterogeneous nature of the clinical condition, the variable plasma levels resulting from the same dose and the similarity of potency for the commonly used antidepressants. Comparison of binding data with *in vivo* pharmacological tests which predict therapeutic drug activities is another possibility. The inhibition of reserpine-induced ptosis in mice is a widely used predictive test for antidepressant activity^{14,15}. A comparison of the inhibition of reserpine-induced ptosis in mice with the inhibition of ^3H -imipramine binding in rats for a series of seven tricyclic and atypical antidepressants (imipramine, desipramine, amitriptyline, chloripramine, iprindole, viloxazine and mianserin) gave a correlation coefficient, r , of 0.588 ($P < 0.25$) (R.R., M.B. and S.Z.L., unpublished results). A larger sample is, however, needed before any conclusions can be drawn. The poor correlation might be due to such factors as metabolic conversion to more or less active forms of the drugs and the possibility that tricyclic and atypical drugs really fall into two classes of which only the former act on the ^3H -imipramine binding site.

Thus, the specific binding of ^3H -imipramine to rat brain membranes is of high affinity, rapid, saturable and possesses a unique selectivity. It is, however, too early to say whether this binding is to an as yet undescribed receptor, as seems to be the case for the benzodiazepines. However, our results suggest that it may be related to the site of action of tricyclic antidepressant drugs, and as such could open up new approaches to the study of the biochemical basis of depression and its treatment with drugs.

A rapid communication of these findings has already been published¹⁶.

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Attempted asymmetric radiolysis of D,L-tryptophan with ^{32}P β radiation

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In 1957 Vester¹ suggested that the then recently discovered^{2,3} violation of parity in weak interactions might be ultimately responsible for the present unique chirality of asymmetric molecules in the biosphere. Since then there has been a growing number of investigations involving the longitudinally polarised electrons from natural β decay. Initial attempts^{4,5} to detect asymmetric effects in several synthetic and degradative organic reactions using a number of β emitters failed and it was not until 1968 that Garay reported⁶ that β radiation from $^{90}\text{Sr}/^{90}\text{Y}$ caused D-tyrosine to degrade more than L-tyrosine in alkaline aqueous solution. Studies have subsequently looked at the possible asymmetric interaction of β rays (or their bremsstrahlung) from a variety of sources (for example ^{106}Rh (ref. 7), $^{90}\text{Sr}/^{90}\text{Y}$ (refs 8, 9), ^{14}C (refs 10–12) ^{32}P (ref. 13) and polarised electrons from an accelerator^{14,15}) with a number of racemic or optically active substrates (usually amino acids). Conflicting results emerging from these and certain related experiments have been tabulated¹³. Perhaps the most striking positive report among these studies is the claim by Darge and coworkers¹³ that D,L-tryptophan (4.9×10^{-7} mol in 2 ml frozen (-25°C) aqueous solution) was 33% decomposed with a 19% relative enrichment of the D-enantiomer in the undestroyed residue by the action during 12 weeks of the β rays from 5 mCi of co-dissolved ^{32}P phosphate. The 33% gross decomposition was estimated from the UV absorption spectrum, and the 19% optical enrichment from the optical activity of the crude diluted reaction mixture, as compared with L-tryptophan at the same concentration ($\sim 10^{-5}$ M). The observed optical rotations used in the latter calculation were minute, -0.0036° for L-tryptophan and $+0.0007^\circ \pm 0.0004^\circ$ (average of 16 observations) for the crude diluted reaction mixture. Because the remarkably large asymmetric effect claimed was based solely on these small observed rotations, and as the results have already been used by others in additional calculations¹⁶, we thought it desirable to attempt to duplicate the observations of Darge *et al.*¹³. We report here the use of a different analytical technique to estimate the enantiomeric composition of the undecomposed residual tryptophan, namely, gas chromatography (GC). The significant advantages of GC over optical rotation for determining the enantiomeric composition of small amounts of an optically active component admixed with impurities have already been described¹⁷.

Two 2-ml aliquots of a solution of D,L-tryptophan (5.1 mg) in 1.8×10^{-3} M HCl (100 ml) were placed in culture tubes and each was treated with 5 mCi (200 μ l) of aqueous $\text{KH}_2^{32}\text{PO}_4$ solution (NEN). A control for each sample was prepared by mixing 2 ml of the above tryptophan solution with 200 μ l 0.042 M KH_2PO_4 . The tubes were sealed and stored at -25°C for 85 days, then thawed and opened. The irradiated samples were each divided into two equal portions and one portion was treated with 20 μ l of a 1.3×10^{-2} M solution of D-tryptophan to allow estimation of the per cent degradation using GC and the enantiomeric marker technique¹⁸. Each solution was then warmed and evaporated in a nitrogen stream and the residues were treated with 1 ml 2-propanol saturated with HCl gas. The culture tubes were sealed and heated at 120°C for 2.5 h, opened and the volatiles were again evaporated in a nitrogen stream. The residues were treated with 1 ml ethyl acetate and 50 μ l heptafluorobutyric anhydride, then were maintained at 120°C for 45 min. The volatiles were again evaporated and each oily residue was dissolved in dichloromethane to give an $\sim 10^{-2}$ M solution for GC analysis. The samples were analysed as previously described¹⁷ using a 46 m $\times$ 0.5 mm (i.d.) stainless steel capillary GC column coated with *N*-docosanoyl-D-valine-*tert*-butylamide phase¹⁹. At 160°C (isothermal) and a N_2 flow rate of ~ 10 ml min^{-1} , baseline GC resolution of the enantiomeric tryptophan derivatives was achieved with the L-isomer eluting in 51.7 min and the D-isomer in 56.9 min. All samples were analysed in replicate, with the control samples interspersed 'back-to-back' with the irradiated samples. The results of these analyses are shown in Table 1, along with the analysis of a known mixture of D- and L-tryptophan which was included to see if an enantiomeric excess (e.e.) as small as $\sim 6\%$ could be reliably detected with such tryptophan derivatives by the GC technique used. A combined GC-mass spectra analysis of the above D,L-tryptophan derivative showed that each GC peak gave the same fragmentation pattern and corresponded to a tryptophan isopropyl ester having one heptafluorobutyryl residue on each nitrogen atom.

Table 1

Exp	1*	1C†	2*	2C†	Known mixture‡
% Degradation	48	—	39	—	—
s.d. ($\pm$)	9	—	9	—	—
% D-tryptophan	50.5	51.1	49.5	50.8	48.3
% L-tryptophan	49.5	48.9	50.5	49.2	51.7
s.d. ($\pm$) ^a	0.6	0.2	0.1	0.3	0.7

* Irradiated samples of D,L-tryptophan.

† Control samples of D,L-tryptophan.

‡ Containing 47.1% D- and 52.9% L-tryptophan.

s.d. Standard deviation.

Although the gross degradation which we observed (43.5% average, Table 1) was somewhat larger than that reported (33%) by Darge *et al.*¹³, we found no evidence whatsoever for any asymmetric radiolysis. While the GC analyses in Table 1 do not seem to be quite as precise as those previously noted¹⁷ for leucine enantiomers, it is nevertheless clear that the irradiated tryptophan residues have an enantiomeric composition not experimentally distinguishable from that of the D,L-tryptophan controls. Furthermore, the detection of a known 5.8% e.e. (as 3.4%) in the known mixture of Table 1 suggests that the $\sim 19\%$ e.e. (59.7% D-, 40.3% L-tryptophan) claimed by Darge *et al.*¹³ could scarcely have been missed by the present GC technique. Unable to find an asymmetric effect after using an alternative analytical procedure (GC), we can only conclude that Darge's reported 19% asymmetric radiolysis of D,L-tryptophan with ^{32}P β radiation may have resulted from a systematic error in the very small polarimetric readings involved or from an artefact present in the crude solutions examined.

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W. Darge*, I. Laczko† and W. Thiemann‡ reply: Bonner *et al.* criticise the reproducibility of our earlier results¹ and claim to have reproduced the original experiment. In fact they have applied a very different analytical technique based on gas-chromatographic analysis of the irradiation residue. There is also the difference that Bonner *et al.* have been able to analyse exclusively the enantiomeric composition of the partially destructed residual tryptophan only, while in our earlier optical rotatory dispersion (ORD) measurements we were able to detect the effect of the sum of all potential chiral products (be they residual tryptophan, intermediate or final irradiation products such as any oxyacids, ketonic, or even peptidic substances commonly encountered in similar experiments). There are possibilities that any such intermediate products exhibit quite specifically large optical rotations and thus contribute to the overall effect in spite of their possibly small concentration in the investigated solution. We believe that this is exactly the advantage of a carefully performed ORD measurement over GC or other analytical methods. Having eliminated 'physical artefacts' as far as possible according to available techniques², one is left with any 'chemical artefacts' in the above defined sense, namely the existence of hitherto unidentified chiral intermediate irradiation products in the chain of the decomposition process of D,L-tryptophan. We never had claimed that any 'stereoselective effect' would manifest itself necessarily in the destruction of racemics but included the equally probable chance of stereoselective synthesis as a source of optical activity.

We wonder why Bonner *et al.* declare our earlier results as 'the most striking positive result': Garay in his original paper³ and Merwitz⁴ had both published much larger 'striking' stereoselective effects as a result of irradiation. Bonner *et al.* themselves confirm the basic hypothesis with their polarised electron bombardment of D,L-leucine⁵. What then is the essential difference between accelerated polarised electrons and β^- particles emitted from radionuclides in this context of 'helicity transferred from the classically physical level to the chemical level'?

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Evidence that three structural genes code for human alkaline phosphatases

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The number of structural gene loci that code for the different molecular forms of human alkaline phosphatase is unknown. Physical properties of the enzymes, immunological data, chemical inhibition and genetic studies suggest that at least three structural genes¹ are involved: one coding for alkaline phosphatase from placenta, another for the enzyme from intestine, and one or more for the enzymes from liver, kidney and bone. Badger and Sussman² have shown that alkaline phosphatases from human liver and placenta are products of different structural genes, and Greene and Sussman³ have shown that alkaline phosphatase from a metastasised bronchogenic carcinoma was nearly identical to the enzyme from placenta. However, other tumour-associated alkaline phosphatases and the enzymes from normal tissues other than placenta and liver have not been identified by conclusive structural criteria⁴, and thus it is not known whether these onco-alkaline phosphatases represent ectopic production or unusual post-translational modification of the enzymes found in normal tissues. We present here, using a sensitive peptide-mapping technique⁵, structural evidence that the enzyme forms from liver, kidney and serum from a patient with Paget's disease of bone (osteitis deformans) are products of the same structural gene and can be easily distinguished from either the intestinal or placental isoenzymes. The technique seems to be useful for the classification of tumour-associated alkaline phosphatases on a structural basis.

The relative pattern of radiolabelled peptides (Fig. 1) clearly shows that there are remarkable structural similarities between the enzymes purified from liver, kidney or Paget's serum. The maps of the radiolabelled peptides of alkaline phosphatase from intestine or placenta are markedly different from each other and from those for the other tissues. Products of different structural genes would be expected to show significant differences in amino acid sequences¹¹ and therefore have different tryptic-peptide compositions. But, allelic proteins of a single structural gene generally have almost identical amino acid sequences¹¹ and therefore would be more difficult to identify on the basis of tryptic-peptide composition. Thus our evidence strongly suggests that only three structural gene loci code for these alkaline phosphatases. The enzymes from intestine or placenta are products of two different loci, and the enzymes found in kidney, liver and in a serum from a patient with Paget's disease of bone, are products of a third locus.

The origin of the elevated serum alkaline phosphatase found in patients with Paget's disease of bone has not been proved, although it is generally considered to be derived from increased osteoblast activity¹². The alkaline phosphatase level as determined by catalytic activity in the Paget's serum used in these studies was 30–40 times the level found in normal sera. The major component of the serum alkaline phosphatase was obtained in homogeneous form in 26% yield and was characterised as 'bone-type' alkaline phosphatase by heat stability, electrophoresis and differential inhibition studies.

Evidence that the stained protein bands were alkaline phosphatase protein was derived from experiments in which heating at 100 °C for 5 min was omitted when samples were prepared for SDS polyacrylamide gel electrophoresis (PAGE). The unheated samples in 1% SDS showed a single enzymatically active, slow-moving band which was coincident with the single protein-stained band⁶. When the protein is heated in 1% SDS in

preparation for SDS-PAGE the enzymatically active band disappears and a faster migrating inactive band appears (equivalent to the subunit molecular weight) (Fig. 2). Peptide maps resulting from either treatment were identical. Also, the loss of enzymatic activity on prolonged incubation at 30 °C in the presence of 1% (w/v) SDS and 5% (v/v) mercaptoethanol was correlated with the disappearance of the slow-moving active band and the appearance of the faster inactive band in the SDS gels⁶. In addition, the peptide map of the protein band from analytical PAGE¹³ of a liver alkaline phosphatase preparation was identical to that obtained by SDS-PAGE. We have also carried out chemical inhibition, electrophoretic, isoelectric focusing and heat stability studies on both the native and desialylated enzymes from each source (unpublished results). We found that these results are entirely consistent with the three structural gene loci hypothesis that is strongly supported by the peptide maps. Furthermore, the properties of the individual enzymes were as would be expected from the appropriate tissue source.

Note that, although the apparent subunit molecular weight (MW) values determined by SDS-PAGE may not be accurate, as the enzymes are glycoproteins and could migrate anomalously on polyacrylamide gels¹⁴, the enzymes from liver, kidney, intestine and Paget's serum all had apparent subunit MWs of ~92,000. The enzyme from kidney was heterogeneous and had subunit MWs of ~96,000, 70,000 and 53,000. However the peptide maps from these three bands suggest that the two lower MW species may have been derived from the larger protein species and thus may represent partially degraded enzyme protein. This degradation may have occurred during the isolation procedure.

The relative spot intensity of two peptide maps from the same preparation is variable. This may be due, at least in part, to variation in the extent of radiolabelling with ¹²⁵I. It is likely that tyrosine residues are selectively iodinated, but diiodotyrosine derivatives as well as iodinated derivatives of phenylalanine and histidine may also be formed¹⁵. Thus peptides may be represented by more than one spot depending on the extent of iodination.

Alkaline phosphatase from various sources may differ in carbohydrate composition. However, the similarity of the peptide maps of alkaline phosphatase from liver, kidney and Paget's serum suggests that differences in carbohydrate composition, if present, have little influence on the resultant peptide map. Elder *et al.*¹⁶ have suggested that the glycopeptides do not migrate in the apolar solvent system used here for thin-layer chromatography. Also our observation that the peptide maps for native and desialylated liver alkaline phosphatase were very similar suggests that the presence of carbohydrate does not greatly influence the results.

The approach used here to classify tissue alkaline phosphatases on a structural basis is suitable for the identification of the enzyme from normal and cancerous tissues. Maps of the radioiodinated peptides can readily be obtained in only a few days using less than 2 µg alkaline phosphatase protein. In addition, it is not necessary to purify the protein to homogeneity provided that it can be separated sufficiently from other proteins by SDS-PAGE (or other techniques) to allow excision of the stained protein band from the gel. Classification of alkaline phosphatases by structural composition will avoid the potential inaccuracies present when classifying gene products on the basis of heat stability, differential inhibition, immunological specificity, electrophoretic mobility or other functional properties.

Another technique used by others to study the structure of alkaline phosphatases is active site labelling with ³²P. Two-dimensional electrophoretic analysis of the labelled enzyme or one-dimensional electrophoresis of the labelled and partially trypsin degraded enzyme have been used to compare the enzymes from milk, placenta, liver, bone and intestine^{17,18}. Active site peptides of alkaline phosphatase have been similarly studied¹⁹. However, this technique is usually applied to impure preparations and makes the assumption that alkaline phosphatase is the only protein that incorporates phosphate in the

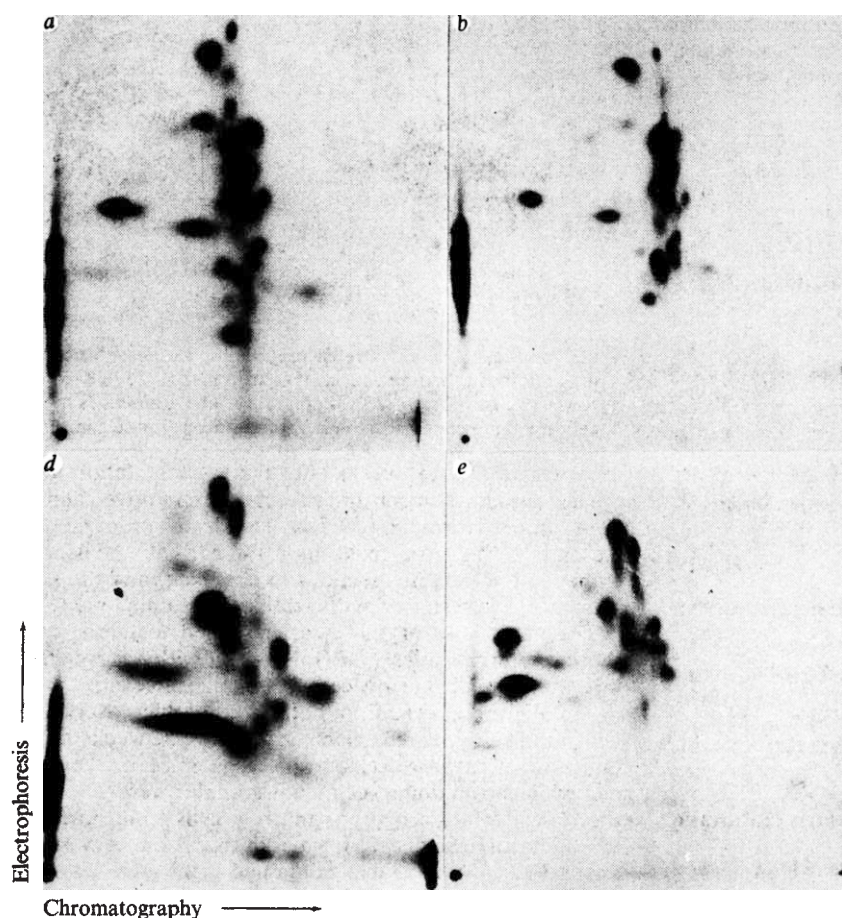


Fig. 1 Comparison of the radioiodinated peptides of human alkaline phosphatases purified from liver (a), kidney (b, 96,000-MW component), Paget's serum (c), intestine (d) and placenta (e). Alkaline phosphatase was purified from human liver, kidney, small intestinal mucosa, placenta, or serum from a patient with Paget's disease of bone as previously described^{6,7}. The purified proteins were subjected to SDS-PAGE⁸ and stained for protein⁹. Gel slices (~1 mm thick) containing the stained protein bands were washed extensively with 25% 2-propanol and then with 10% methanol, and dried under a stream of nitrogen. The proteins within the gel slice were then radioiodinated with ¹²⁵I (Edmonton

Pharmaceutical, specific activity ~17 Ci mg⁻¹) by a modification of the chloramine T method¹⁰ as described by Elder *et al.*⁵. Each gel slice was dried under a stream of nitrogen and incubated in 0.5 ml of 50 µg ml⁻¹ trypsin (Sigma T-1005, 7,500 U mg⁻¹) in 50 mM NH₄HCO₃ buffer (pH 8.0) for 16 h at 37 °C, after which the supernatant was lyophilised. The tryptic digests were then analysed by thin-layer electrophoresis followed by thin-layer chromatography in a second direction. The lyophilised samples were dissolved in 20 µl of solution A (acetic acid/formic acid/water, 15:5:80) and ~1 × 10⁶ c.p.m. (1–5 µl) were spotted onto 20 cm × 20 cm cellulose-coated TLC plates (Eastman Kodak). The plates were moistened with solution A and electrophoresed at 1,000 V for ~1 h at 8 °C. The plates were dried and chromatographed in a second direction using a solvent mixture containing butanol/pyridine/acetic acid: water (65:50:10:40). Radioactivity was detected by autoradiography using Kodak XR-1 X-ray film with an intensifying screen (Dupont, Cronex par speed) and exposure at -70 °C for 4 h, or as required.

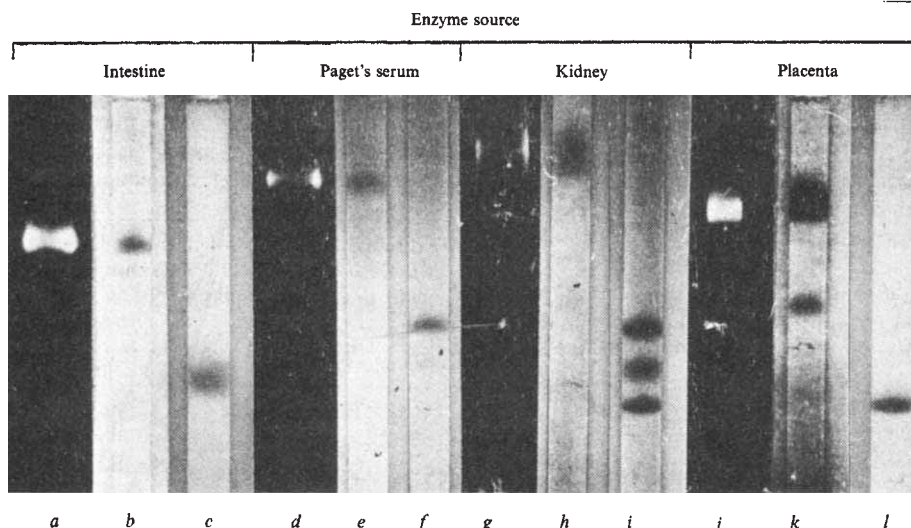


Fig. 2 Purity of alkaline phosphatases from human tissues. Alkaline phosphatases purified from small-intestinal mucosa, Paget's serum, kidney or placenta were subjected to SDS-PAGE⁸ in denaturing and non-denaturing conditions. Identical criteria of purity for the enzyme from liver have been previously presented⁶. Non-denaturing conditions were identical to denaturing conditions except that heating the sample in 1% SDS to 100 °C for 5 min was omitted; instead, the sample in 1% SDS was left at room temperature for 5 min. a, d, g, j, Samples were prepared in non-denaturing conditions, electrophoresed, stained for enzyme activity⁶, photographed and then (b, e, h, k) stained for protein⁹. c, f, i, l, Samples were prepared in denaturing conditions, electrophoresed and stained for protein⁹. Samples not denatured showed an enzymatically active band which corresponded to a protein-stained band. Two protein-stained bands were evident for nondenatured placental enzyme and both of these were active. In denaturing conditions samples from intestine, Paget's serum or placenta showed a single protein-stained band and the enzyme from kidney showed three bands (see text). For any one tissue denatured and non-denatured protein gave identical peptide maps. The two faster migrating bands in the kidney sample appeared to be proteolytic degradation products.

experimental conditions. Furthermore, the technique does not give detailed structural information about the entire molecule.

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Clonal nature of mast-cell clusters formed in W/W^v mice after bone marrow transplantation

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We have recently found that the number of mast cells in the skin of adult W/W^v mice is less than 1% of that observed in congenic $+/+$ mice, and that no mast cells are detected in other tissues of W/W^v mice¹. After the transplantation of bone marrow cells from congenic $+/+$ mice, the number of mast cells in the skin, stomach, caecum and mesentery of the W/W^v mice increased to levels similar to those of the $+/+$ mice¹. Study of the mast-cell number in the W/W^v mice at various times after transplantation suggested to us that mast cells might develop in groups, particularly in the skin and mesentery. In this report, we have attempted to elucidate the possible clonal origin of such mast-cell clusters from a single precursor cell, using giant granules of beige ($C57BL/6-bg^1/bg^1$, Chediak-Higashi syndrome) mice as a marker to identify the origin of the mast cells^{2–4} (Fig. 1). We found that when $WB-W/+ \times C57BL/6-W^v$ ($WBB6F_1$)- W/W^v mice were injected with a mixture of bone marrow cells from beige $C57BL/6$ mice and normal $C57BL/6$ mice, more than 95% of mast-cell clusters consisted of either beige-type cells alone or normal-type cells alone. We conclude, therefore, that the cluster of mast cells originated from a single precursor cell.

Three-month-old $WBB6F_1$ - W/W^v mice were injected with bone marrow cells from either beige $C57BL/6$ mice (2×10^7) or normal $C57BL/6$ (2×10^7), or with a mixture of bone marrow cells from beige $C57BL/6$ mice (10^7) and normal $C57BL/6$ mice (10^7).

On various days after the cell injection, a blood sample was obtained from a lateral tail vein of W/W^v recipient mice, and the number of erythrocytes was counted with a haemocytometer. The smears were fixed in formaldehyde vapour, stained with Sudan Black B and counterstained with Giemsa solution^{5,6}. One hundred neutrophils with distinctly segmented nuclei were scored for the presence or absence of giant sudanophilic granules.

A biopsy of the dorsal skin of the W/W^v recipient mice was carried out 10 weeks after bone marrow transplantation, and the

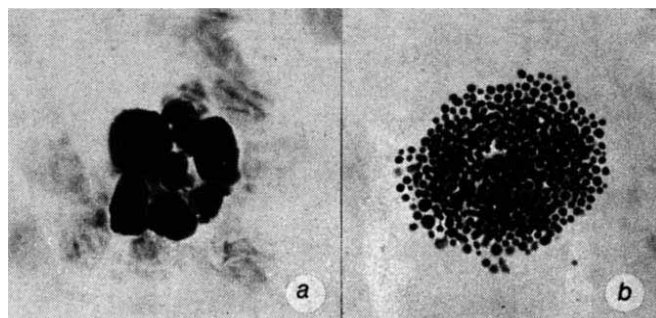


Fig. 1 Mast cells in a stretch preparation of mesentery, stained with acidified toluidine blue. *a*, Mast cell of beige $C57BL/6$ mouse; *b*, Mast cell of normal $C57BL/6$ mouse. The granules in the beige cell are significantly increased in size and decreased in number.

mice were killed 15 weeks after the transplantation. Pieces of the dorsal skin, stomach and caecum were removed and fixed in 10% buffered formalin (pH 7.2). The stretch preparation of the mesentery was also fixed in 10% formalin. Tissues were embedded in paraffin; sections (5 μ m thick) and the stretched mesentery were stained with acidified toluidine blue.

The number of erythrocytes increased to levels similar to those of normal mice ($WBB6F_1-+/+$) within 6 weeks of the injection of bone marrow cells from beige mice or normal mice, or the mixture of cells from both types of mice. About 80% of neutrophils contained giant granules 15 weeks after the injection of marrow cells from beige mice. In contrast, no neutrophils containing such giant granules were detected after the transplantation of bone marrow cells from normal mice. When the mixture of cells from both types of mice was injected, about 30% of neutrophils contained giant granules 15 weeks after transplantation.

Clusters of mast cells were detected in the sections of the skin removed by biopsy 10 weeks after the cell injection (Fig. 2*a*). All clusters consisted of mast cells containing giant granules alone when bone marrow cells of beige mice had been injected, whereas they were all composed of mast cells containing granules of regular size when the cells of normal mice had been used. When the mixture of cells from both types of mice was transplanted, about half of the clusters contained beige-type mast cells alone and the other half contained only normal-type mast cells. Although very few clusters were observed to contain both types of cells, one type of cell was predominant (more than 90% of total) in all cases. Serial sections of the skin revealed that the type of mast cells did not change in different parts of the same cluster.

In the skin, stomach and caecum of $WBB6F_1$ - W/W^v mice killed 15 weeks after the injection of mixed marrow cells, it was

Table 1 Constitution of mast-cell clusters detected in the mesentery of $WBB6F_1$ - W/W^v mice 15 weeks after the injection of a mixture of bone marrow cells from beige $C57BL/6$ mice and normal $C57BL/6$ mice

Mouse no.	No. of mast-cell clusters of each type			Total
	Pure beige	Pure normal	Mixed	
1	4	10	0	14
2	11	10	2	23
3	6	12	3	21
4	3	2	0	5
5	5	4	0	9
6	6	7	0	13
7	4	7	0	11
8	31	17	1	49
9	6	3	1	10
10	1	1	0	2
11	3	2	0	5
12	21	13	0	34
Total	101	88	7	196

The method of bone marrow transplantation has been described previously¹.

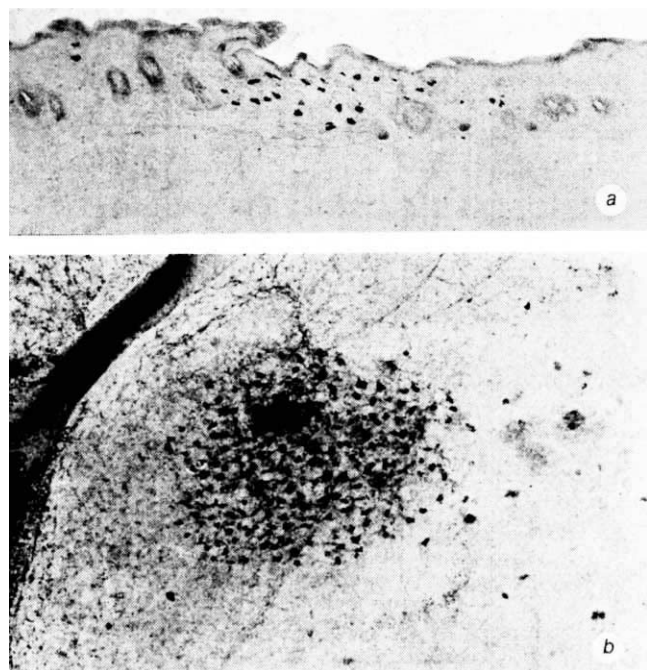


Fig. 2 *a*, A mast-cell cluster in the skin of a WBB6F₁-W/W^v mouse detected 10 weeks after bone marrow transplantation. A group of more than 10 mast cells in one section (5 µm thick) was defined as a cluster. *b*, A mast-cell cluster in the mesentery of a WBB6F₁-W/W^v mouse detected 15 weeks after the bone marrow transplantation. A discrete group of more than 20 mast cells in the stretched mesentery was defined as a cluster.

rather difficult to find discrete clusters because of the diffuse development of the mast cells. However, only one type of mast cell seemed to predominate in any given area of tissue.

In contrast with other tissues, discrete clusters of mast cells were observed in the stretched mesentery 15 weeks after bone marrow transplantation (Fig. 2*b*), located mainly in the fat tissue near blood vessels. As all cells contained in a cluster could be scored in the stretched mesentery without tedious examination of serial sections, the type and number of mast cells in each cluster were determined. As shown in Table 1, 189 out of 196 clusters (96%) consisted of either beige-type mast cells or normal-type mast cells. The largest cluster contained about 300 cells.

The present results demonstrate that mast-cell clusters which appeared in the skin and mesentery of W/W^v mice after the transplantation of bone marrow cells are derived from a single precursor cell. This is consistent with our recent observation that the precursor of the mast cell migrates through the bloodstream⁷ and proliferates at the site of differentiation⁴. The bone marrow-derived precursor cell seems to produce up to 300 progenies when it differentiates into mast cells in the mesentery.

We have recently found that mast cells are as severely decreased in number in the *Sl/Sl^d* mice as in W/W^v mice⁸. In contrast to W/W^v mice, the number of mast cells in *Sl/Sl^d* mice did not increase after the transplantation of bone marrow cells from congenic +/+ mice⁸. The injection of bone marrow cells from *Sl/Sl^d* mice increased the number of mast cells in the skin, stomach, caecum and mesentery of W/W^v mice. This indicates the presence of mast-cell precursors in the bone marrow of *Sl/Sl^d* mice⁸. The failure of haematopoietic colony formation in the spleen of *Sl/Sl^d* mice⁹ and the failure of melanocyte differentiation in the skin of the same mutant mice¹⁰ are attributed to a defect in the microenvironment¹⁰⁻¹². The development of mast-cell clusters also seems to be under the control of the microenvironment.

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Interaction of C-reactive protein with artificial phosphatidylcholine bilayers

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C-Reactive protein (CRP), the most characteristic of the 'acute phase proteins' (ref. 1) is thought to participate in the mediation and/or modulation of acute inflammatory processes, but its exact function is unknown. CRP has a Ca²⁺-dependent binding specificity for phosphorylcholine,² the polar head group of two widely distributed lipids, lecithin (phosphatidylcholine, PC) and sphingomyelin (SM). A number of observations³⁻⁵ suggest that at least some of the biological activities of CRP depend on its interaction with phospholipids of cell membranes. In addition, interaction of CRP with PC- and SM-containing lipid dispersions⁶ and with PC-containing liposomes⁷ can activate the complement system. We report here that binding of CRP to model membranes of PC requires the incorporation into the bilayer of lysophosphatidylcholine (LPC). Thus, a disturbance of the molecular organisation of the bilayer appears to be necessary for binding of CRP. These findings provide a possible biochemical explanation for binding of CRP to damaged but not intact cell membranes³ and might be relevant to its biological function.

Binding of CRP to multilamellar PC-liposomes and single-walled PC-vesicles was studied by molecular sieve chromatography on agarose columns. CRP was purified from ascitic fluid by a recently described method⁸ and labelled with ¹²⁵I by the lactoperoxidase method⁹. PC from egg yolk, LPC and phosphatidic acid (PA) were prepared as described previously¹⁰. Their purity was ascertained by thin layer chromatography on silica gel H. In initial experiments the binding of CRP to multilamellar liposomes and single-walled vesicles was examined. To this end, lipid dispersions consisting of PC, cholesterol and PA (77:15:8 mol%) were sonicated for short periods and after incubation with ¹²⁵I-CRP were subjected to chromatography on Sepharose-4B columns. As shown in Fig. 1, two peaks of phospholipid were obtained: one containing large multi-layered liposomes corresponding to the void volume of the column, and a second containing small single-walled vesicles¹¹. ¹²⁵I-CRP eluted from the column as a separate single peak at the same position as unbound protein. In addition, binding of ¹²⁵I-CRP was tested with neutral, negatively charged (10 mol% PA) and positively charged (10 mol% stearylamine) PC-vesicles by subjecting the CRP-vesicle mixture to chromatography on Bio-Gel A-0.5m columns. In repeated runs, the phospholipid and the protein eluted from the column as distinctly separate peaks. Only a small amount of the eluted

^{125}I -CRP (1–13%) was occasionally found associated with the vesicle peak. Identical results were obtained with dimyristoyl-PC and also when up to 40% cholesterol was incorporated into negatively charged vesicles. Similarly, when unlabelled CRP was substituted for ^{125}I -CRP only 10% of the eluted protein was associated with the vesicle peak. In these experiments, the concentration of CRP in the eluted fractions was determined by measuring intrinsic tryptophan fluorescence.

On the basis of these data we conclude that CRP cannot form a complex with the polar head group of PC present in an artificial bilayer. Given the known binding affinity of CRP for phosphorylcholine¹², these data could be interpreted to indicate that in intact PC bilayers the polar head groups are not freely accessible to the protein. This interpretation seems to contradict available models of the structure of phospholipid bilayers which indicate that polar head groups are exposed to the external aqueous phase and thus available for reaction. However, NMR data on artificial PC-bilayers have demonstrated the presence of intramolecular interactions between polar head groups restricting the extent of their accessibility to the water phase¹³. Furthermore, studies using human red cells revealed that hydrolysis of phosphoglycerides by phospholipase C required the presence of surfactants¹⁴, indicating that the polar head groups were not freely accessible to this enzyme in the intact bilayer. These considerations led us to test the effect of detergent incorporation into the PC-bilayers on the binding of CRP. Considerable amounts of detergent can be incorporated into PC bilayers without disrupting them¹⁵, and up to 50 mol% of LPC, Triton X-100 or SDS does not cause a breakdown of the bilayer structure. On the other hand, incorporation of detergent results in changes in a number of the known properties of the bilayers including changes in permeability¹⁶, transition temperature¹⁷ and susceptibility to hydrolysis by lipolytic enzymes¹⁴.

The effect of incorporating increasing amounts of LPC into PC-vesicles on the binding of ^{125}I -CRP is shown in Fig. 2. At a molar ratio of PC:LPC 2:1, 83% of the eluted ^{125}I -CRP was found associated with the phospholipid peak. The ratio of radioactivity to lipid phosphorus was constant across the peak, indicating homogeneous binding of the protein to the vesicles. This shows that the membrane interface formed by PC and LPC exposes the phosphorylcholine group to the extent that CRP can

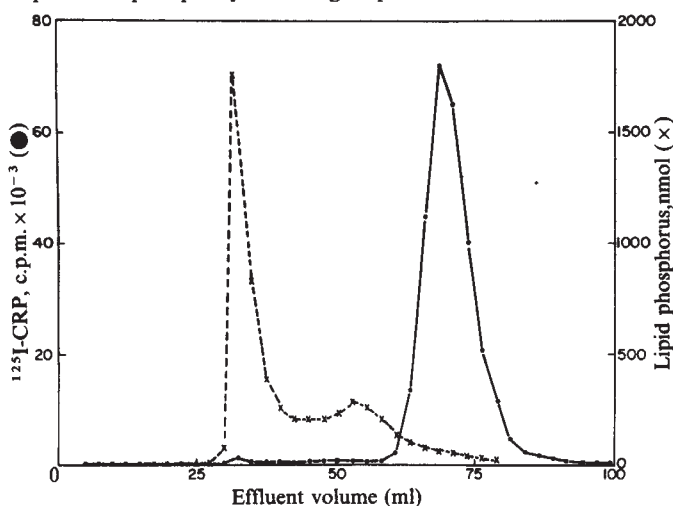


Fig. 1 Gel filtration of a mixture of ^{125}I -CRP and PC-vesicles using Sepharose 4B. Vesicles were prepared by suspending 55 μmol egg-PC, 11 μmol cholesterol and 5.5 μmol PA in 5.0 ml of 10 mM Tris-HCl, 150 mM NaCl, 2 mM CaCl_2 , pH 7.2. The dispersion was subjected to sonic irradiation for 10 min at 0°C under a stream of nitrogen, using a Branson tip sonifier at 70 W. Vesicles (6.6 μmol PC) were incubated with 0.56 nmol ^{125}I -CRP in a total volume of 0.8 ml for 30 min at room temperature. The mixture was then applied on a 1.5×45 cm column of Sepharose 4B equilibrated in 10 mM Tris, 150 mM NaCl, 2 mM CaCl_2 , pH 7.2. Phospholipid content of fractions is expressed as lipid phosphorus, measured by the method of Rouser *et al.*¹⁹. The recovery of applied protein and lipid was 78% and 75% respectively.

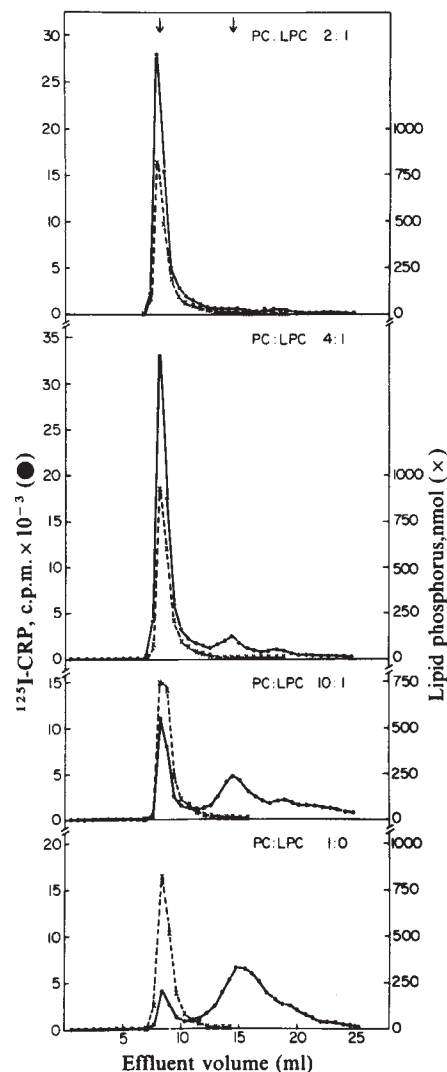


Fig. 2 Gel filtration of mixtures of ^{125}I -CRP and PC-vesicles containing various amounts of LPC, using Bio-Gel A-0.5 m. Vesicles were prepared from a chloroform solution of a mixture of egg-PC and egg-LPC at the indicated molar ratios. The lipid was dried by rotary evaporation under reduced pressure and dispersed in 10 mM Tris-HCl, 150 mM NaCl, pH 7.2 at a concentration of 12 $\mu\text{mol ml}^{-1}$. The dispersion was sonicated under nitrogen at 0°C until it became clear. Sonication time varied from 10 min for the PC:LPC 2:1 vesicles to 45 min for the PC vesicles. The vesicle preparations were then centrifuged at 160,000g for 30 min to remove metal particles and large lipid aggregates. Vesicles (2.6 μmol of phospholipid) were incubated for 30 min at room temperature with 0.23 nmol ^{125}I -CRP in a total volume of 315 μl in buffer containing 9.5 mM CaCl_2 . The mixture was then applied on a 7×45 cm Bio-Gel A-0.5 m column equilibrated and eluted with 10 mM Tris-HCl, 150 mM NaCl, 2.5 mM CaCl_2 , pH 7.2. Mean recoveries $\pm$ s.e.m. for ^{125}I -CRP and phospholipid were $67 \pm 4\%$ and $79 \pm 3\%$, respectively. The arrows indicate elution volumes of blue dextran (left) and ^{125}I -CRP (right).

form a regular complex. Increasing the molar ratio of PC:LPC resulted in a decrease of the percentage of vesicle-bound ^{125}I -CRP with a concomitant increase of the free ^{125}I -CRP peak. Thus, the percentage of eluted ^{125}I -CRP found associated with vesicles containing PC:LPC at molar ratios of 4:1, 10:1 and 1:0 (no LPC) was 73, 34 and 12%, respectively.

To examine whether the observed binding of CRP to the vesicles was an exclusive function of LPC or could also be reproduced with other surfactants, three other surfactants were also examined. Figure 3 shows that Triton X-100 (Rohm and Hass), or sodium deoxycholate (DOC, Merck) incorporated into egg-PC-vesicles at molar concentrations of 45% did not promote the binding of ^{125}I -CRP to the vesicles. The experiment with Triton X-100 was repeated by equilibrating and eluting the

column in buffer containing 0.35 mM Triton X-100, which is slightly above its critical micellar concentration, to avoid dissociation of the surfactant from the vesicles during the chromatography¹⁸. Again, no binding of ¹²⁵I-CRP to the vesicles was observed. However, when sodium dodecyl sulfate (SDS, Merck) was incorporated into PC-vesicles at the same molar concentration of 45%, some binding of ¹²⁵I-CRP was observed. As shown in Fig. 3, 38% of the eluted protein was associated with the SDS-containing vesicles. This compares to a binding of 84% for the PC-vesicles that contain LPC at a molar concentration of 50%.

The present data demonstrate that LPC is by far the most effective surfactant in promoting the binding of CRP to PC vesicles. We do not yet know whether binding occurs to the polar head group of LPC or to that of LPC and PC. The failure of Triton X-100 and DOC to promote binding raises the question of whether derangement of the molecular organisation of the PC bilayer is a prerequisite for binding. On the other hand, different surfactants are known to interact differently with phospholipid bilayers, the mode of interaction depending on such factors as the overall conformation, size, charge and length of acyl chain¹⁵. In terms of the possible biological implications of the present observation, previous data on the *in vivo* deposition of CRP are relevant. Kushner and Kaplan³ reported that at experimental inflammatory sites CRP could be demonstrated in close association with membrane structures of altered or necrotic but not of normal cells. Thus, an alteration or complete breakdown of the natural orientation of cell membranes appeared to be necessary for *in vivo* binding of CRP, although the nature of the CRP-binding moieties was not determined. However, considering the two sets of data together, it seems reasonable to specu-

late that following tissue injury or necrosis, CRP can bind to phosphorylcholine-containing phospholipids of necrotic or altered cell membranes. As previously suggested⁶, such binding could lead to complement activation and generation of chemotactic and opsonic factors, resulting in attraction of phagocytic cells and enhanced phagocytosis and thus contributing to the repair of the injured site. This hypothetical sequence of events should be considered as a possible biological function of CRP.

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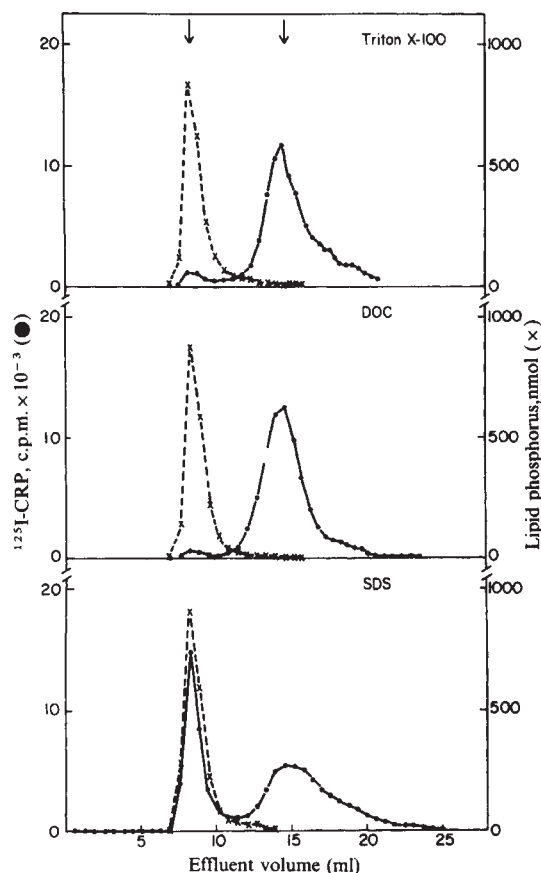


Fig. 3 Gel filtration of mixtures of ¹²⁵I-CRP and PC-vesicles containing various surfactants, using Bio-Gel A-0.5 m. Vesicles were prepared by dispersing egg-PC ($11 \mu\text{mol ml}^{-1}$) in 10 mM Tris-HCl, 150 mM NaCl, pH 7.2 containing $5 \mu\text{mol ml}^{-1}$ of the respective surfactant. Each dispersion was sonicated until it became clear (3-5 min). Other experimental details were as for Fig. 2. Mean recoveries $\pm$ s.e.m. for ¹²⁵I-CRP and phospholipid were $82 \pm 4\%$ and $88 \pm 1\%$, respectively.

Evidence for the bilobal nature of diferric rabbit plasma transferrin

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Plasma transferrin is involved in iron transport within the circulatory system of vertebrates, and provides an iron source for haemoglobin synthesis and other metabolic requirements. However, despite extensive studies by spectroscopic, biochemical and physiological techniques, the nature of iron binding and the mechanisms of uptake and release of iron are not fully understood. Plasma transferrins¹ are monomeric glycoproteins with a molecular weight of approximately 80,000 (ref. 2); they have two similar and very strong binding sites for Fe(III), together with two associated anion binding sites. Fragmentation studies on various transferrins³⁻⁶ have shown that the polypeptide chain is composed of two domains formed from the N-terminal and C-terminal halves of the polypeptide chain. Each domain contains one metal binding site. The marked sequence similarities which exist between the two halves may reflect a doubling of an ancestral structural gene during the phylogenetic development of the protein^{2,7}. Preliminary crystallographic investigations of diferric rabbit plasma transferrin have been reported from this laboratory⁸. We now report initial studies of the X-ray structure determination of diferric rabbit plasma transferrin which have led to a 6-Å resolution electron density map.

The protein crystallises in the space group $P4_12_12$ or the enantiomorph, with $a = b = 127.5(3) \text{ \AA}$, $c = 145.4(3) \text{ \AA}$, and one molecule per asymmetric unit. The native crystals are grown within the temperature range of 4-8 °C; at higher temperatures

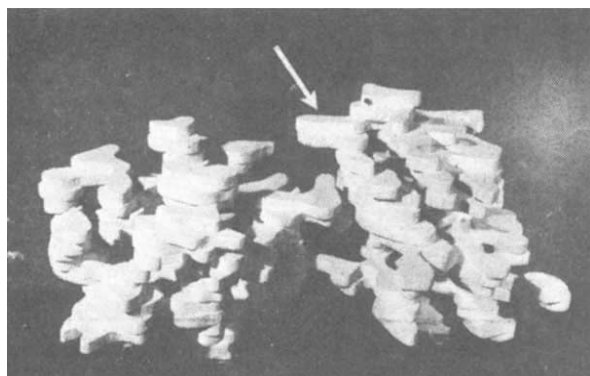


Fig. 1 A balsa wood model of electron density taken to represent one molecule of diferric rabbit plasma transferrin. The bilobal nature of the molecule is clearly seen and the arrow indicates the region of high electron density in the right hand lobe which may be α -helix. The prominent cleft in this lobe lies immediately below this region. The common platinum/mercury site is at the top of the left hand lobe.

they dissolve. Sixty-eight per cent of the crystal volume is occupied by solvent. The crystals are unstable in the absence of the mother liquor and derivatives were prepared by soaking the native crystals in solutions of heavy atom reagents in the mother liquor. The soaking conditions for the three derivatives used to produce the map are given in Table 1.

X-ray diffraction data for an octant of reciprocal space were collected on a Hilger and Watts Y290 automated four-circle diffractometer for the native crystals and three isomorphous derivatives. The diffractometer was equipped with a helium path, and a low-temperature device and Ni-filtered copper radiation were used throughout. Data were collected at 5°C using ω -step scans. Three or four crystals were required per data set and intensities were estimated by a 'moving window' technique⁹; absorption corrections were made according to the method of North *et al.*¹⁰.

Difference Patterson syntheses computed with ΔF_{iso}^2 (where ΔF_{iso} is the isomorphous difference) as coefficients enabled two major heavy atom sites to be located for the mercury derivative and one site for the platinum derivative. Only a partial interpretation of the uranyl map was possible at this stage. The three major uranyl sites were located from difference maps computed with protein phases obtained by multiple isomorphous replacement using the refined mercury and platinum positions. In addition, the heavy atom positions of the mercury and platinum derivatives were checked by the use of cross-difference Fourier syntheses based on single isomorphous protein phases from the uranium positions.

For each derivative the heavy atom coordinates and relative occupancies were refined against ΔF_{iso} for the centric reflections. Isotropic temperature factors were held constant at a value of 20 Å. Difference electron density maps were computed with coefficients $(\Delta F_{\text{iso}} - F_{\text{Hcalc}})e^{i\alpha_{\text{Hcalc}}}$ (where F_{Hcalc} and α_{Hcalc} are the calculated amplitude and phase of the heavy atom structure factor), but only revealed significant minor sites for the uranyl derivative.

The final heavy atom parameters and *R* factors are presented in Table 2. Protein phases were calculated from these parameters using the method of multiple isomorphous replacement with all three derivatives in space group $P4_12_12_1$.

Table 1 Derivatives used to produce the electron density map

Heavy-atom reagent	Time of soaking	Concentration	Method
Potassium chloroplatinate	36 h	7 mM	Direct soaking
Mercuric chloride	24 h	8 mM	Dialysis cell
Uranyl acetate	9 d	10 mM	Dialysis cell

Data collected to 6 Å.

Table 2 Heavy atom parameters and refinement statistics

Site	x	y	z	Z	B(Å ²)
Mercuric chloride					
1	0.487	0.125	0.178	0.086	20
2	0.032	0.278	0.185	0.046	20
<i>R</i> (centric) = 0.61					
Potassium chloroplatinate					
1	0.024	0.285	0.184	0.063	20
<i>R</i> (centric) = 0.58					
Uranyl acetate					
1	0.307	0.257	0.090	0.032	20
2	0.214	0.495	0.090	0.029	20
3	0.633	0.391	0.013	0.020	20
4	0.756	0.066	0.069	0.010	20
5	0.458	0.315	0.075	0.014	20
<i>R</i> (centric) = 0.62					

x, y, z are the heavy atom fractional co-ordinates. Z is relative occupancy. All site occupancies are on the same arbitrary scale. $\langle |\alpha_P - \alpha_H| \rangle$, the mean absolute difference between the protein and heavy atom phase angles, is 86.9°, 89.3° and 90.7°, respectively, for mercuric chloride, potassium chloroplatinate and uranyl acetate.

The mean overall figure of merit for the 3,226 reflections is 0.50.

The 'best' phases and figures of merit¹¹ were used to calculate a 6-Å resolution electron density map. This map shows a well defined volume of high electron density with approximate maximum dimensions of 95 × 60 × 50 Å. This is represented by a balsa wood model (Fig. 1) which defines the molecular boundary. The high solvent content of the crystals and the low salt concentration of the liquid of crystallisation proved useful in this respect. The model shows that the molecule consists of two lobes of roughly equal size. The major axes of these lobes are inclined at about 30° to one another and their total volume is consistent with a protein content of about 30% in the crystal. All the heavy atom sites lie close to the surface of the molecule. Four of the uranyl sites and the major mercury site are on one lobe, and the remaining uranyl site and the common platinum and mercury site, which may correspond to the position of a methionine residue, are on the second lobe. The former lobe exhibits a pronounced cleft in the region of the join between the two lobes and close to this feature there is a column of high electron density which could be α -helix. On the opposite side of this same lobe another strong region of electron density may prove to be β -sheet at higher resolution. The second lobe also has a cleft, although less pronounced, close to the join.

The two lobes of the molecule may correspond to the two domains, each containing an iron binding site, proposed from fragmentation studies. However, the locations of the iron binding sites and the arrangement of amino acid residues in the diferric rabbit plasma transferrin molecule must await study at higher resolution. The collection of X-ray data to 3.0 Å resolution, the practical limit of the diffraction pattern using conventional sources, is now in progress.

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reviews

Shaping psychology

O. L. Zangwill

Pioneers of Psychology. By R. E. Fancher. Pp. 397. (W. W. Norton: New York, 1979.) \$15.95.

THE author tells us in his Preface that this book is about "the lives and works of some of the people who have shaped the modern science of psychology." Although there are still some people who hold, with William James, that psychology is less a science than the hope of a science, it can safely be said that the people Dr Fancher writes about, whether they were primarily philosophers or physicists, physiologists or physicians, have had much to do with the shaping of psychology as we know it today. His account even extends to a few, such as Wilhelm Wundt, who were essentially experimental psychologists even if the posts they occupied fell within other academic disciplines, and to two contemporary "pioneers", Jean Piaget and B. F. Skinner. Nevertheless, it is essentially an outline, somewhat superficial though extremely readable, of the background of modern psychology.

The opening chapter, on Descartes, sets the tone of the book. This may be described as a blend of thumb-nail biography and the history of ideas. As Dr Fancher right points out, Descartes' knowledge of anatomy was amateur even by the standards of his time and his physiology was mostly wrong. Nevertheless, his concept of reflex action and the mechanistic model of the nervous system which it inspired has largely animated the growth of neurophysiology and provided experimental psychology with its basic philosophy. True, Descartes' resolution of the mind-body problem has left many people unsatisfied but it has nevertheless remained an influential belief not only among philosophers but many scientists too. Cartesian dualism may well be wrong but it is inseparable from the evolution of modern science.

The next chapter is headed "The Physiology of Mind" and traces the development of our ideas about brain function from Gall's phrenology to present-day neurosurgery. Inevitably, Dr Fancher deals at some length with Pierre Flourens, whose work on the effects of brain lesions on behaviour in animals did much to discredit phrenology and promote the view that sensory and motor functions lack discrete representation in

the cerebral cortex. As has often been pointed out, his conclusions in many respects anticipated those of Karl Lashley over a century later. Indeed it was the clinicians rather than the experimental physiologists who changed the whole climate of localisation theory. Following the claims of Broca and others that supposedly circumscribed disorders of language might follow localised cerebral lesions, a kind of neo-phrenology evolved envisaged in terms not of bumps but of brain centres. Although the concept of a brain centre was soundly based on the theory of reflex action, it became only too readily contaminated by psychology. The result was a new mythology of cerebral functions bearing little relation either to the mechanisms of nervous action or to the realities of human behaviour.

It can perhaps be said that neurology is now working towards a less extreme conception of the cerebral localisation of psychological function. Although focal deficits resulting from cortical lesions have been charted with a very fair degree of precision, we still lack any clear conception of the relation of deficit to normal function and the extent to which the machinery of the brain may be inferred from studies of its breakdown. Interestingly, Dr Fancher views Penfield's work on the effects of cortical stimulation in conscious man as the latest and most decisive phase in the localisation controversy. Brilliant indeed as was Penfield's work, it cannot really be said to have made a major theoretical contribution to solution of the mind-body problem. Indeed, Roger Sperry's work on 'split-brain' man might seem to offer greater hope of a fresh consideration of the physical basis of mind and consciousness. It is a pity that Sperry's work fails to find mention in Dr Fancher's book.

We then come to two chapters mainly concerned with the origins of modern experimental psychology. The first deals principally with studies of human sensation and perception and centres on Helmholtz: the second with Wilhelm Wundt, whom the author places in sharp contrast with William James, somewhat to Wundt's disadvantage. In connection with Helmholtz, Dr Fancher does well to place stress on the Kantian influence on his thinking, which his strongly empiricist bias has caused many to disregard. It is evident, for example, that, although Helmholtz laid great emphasis on the role

of experience and learning in human perception, more especially the perception of space, he treated sensation as basically innate. Whether we regard Helmholtz as a physicist, a physiologist or a psychologist is irrelevant. Though he did not himself wish to be regarded as a psychologist, there can be no doubt that, without him, human experimental psychology would never have found its feet.

This inevitably brings us to Wundt, who is remembered as the founder of the first laboratory in the world exclusively dedicated to experimental psychology. Indeed it celebrates its centenary this year. James was sceptical as to its promise: As he tartly remarked, "there is little of the grand style about these new prism, pendulum and chronograph philosophers." Yet, to give him his due, Wundt did try to bring together psychophysical studies and the physiology of the senses, and his work enabled experimental psychology to emerge as an independent discipline. Yet James has perhaps been proved right in so far as experimental psychology, though nowadays respectable, is seldom scintillating.

The scene now shifts to a very different theme, remote from the University but close to the consulting room. This is the theme of psychological medicine, and its origins are traced to the early hypnotists beginning with Mesmer and culminating in Freud. On Mesmer, Dr Fancher has disappointingly little new to tell us. In particular, he throws no light on Pattie's contention that his doctoral thesis was cribbed almost word-by-word from a thesis by one Richard Mead, an obscure Irish physician. This claim, if true, detracts considerably from the more sympathetic judgements of Mesmer's personal integrity advanced by several recent biographers. On Freud, the subject of an earlier book by the same author, Dr Fancher is knowledgeable and sympathetic. His chapter is of considerable value in stressing the importance of much of Freud's earlier work, in particular his draft project for a scientific psychology, written in 1895 but published only several years after his death, and the early case studies on hysteria. His book on *The Interpretation of Dreams* rightly receives high commendation. It is disappointing, however, that Dr Fancher sees no obvious way whereby the insights of psychoanalysis might be linked with the sterner disciplines of the neurosciences.

The only reference to British psychology is a chapter on Francis Galton and the psychology of individual differences. This once again covers familiar ground and it is a pity that a recent biography by D.W. Forrest, altogether more digestible than Pearson's monumental three volumes, fails to find mention. Fancher's short account is useful in stressing Galton's fascinating studies of visual imagery and word-association, which have been relatively neglected in recent years, and he deserves credit for not getting too embroiled in the nature-nurture controversy, so productive of emotion though hardly of wisdom in contemporary psychological discussion.

The last two chapters bring us closer to the modern scene. The penultimate chapter gives an account of Pavlov's work followed by a short outline of the history of behaviourism. Although the account of J. B. Watson's life and work is not without historical interest, it fails to bring out fully how little behaviourism, in its early days at least, owed to Pavlov (it was more influenced by his rival Becterev); nor how greatly Pavlov himself detested behaviourism, which he regarded as little better than the mentalistic psychology it

replaced. The importance of Lashley as a co-founder of behaviourism is insufficiently stressed. The final chapter contrasts Jean Piaget with B. F. Skinner, both described as modern pioneers. Only the future will show.

Although much of this book has been compiled from secondary sources and makes no advance on the widely accepted version of the history of experimental psychology which we owe to the late E. G. Boring, this book can be recommended for its thoughtful and well-written account of some important threads in its more recent phases of development. It is only perhaps in the sections dealing with German psychology that the author fails in some measure to catch the spirit of the age, due to the probability that he is unable to read German. Although it may be true that English is now the accepted language of European science, the historian of science would do well to acquire at all events, a reading knowledge of the mother tongue of European scientists. □

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Free radical chemistry

Radicals. By D.C. Nonhebel, J.M. Tedder and J.C. Walton. Pp. 200. (Cambridge University Press: Cambridge, 1979.) Hardback £14; paperback £5.50.

To cover adequately the many important aspects of free radical chemistry in approximately 200 pages, even at the undergraduate level, is an impossible task. This book makes an excellent attempt at the impossible.

The book is aimed at honours and post-graduate chemists but should also be useful to chemists at all levels in need of background information on radical chemistry. The coverage is wide-ranging although the depth of treatment is variable. Almost no original references are quoted but there is an extensive list of books and reviews for each chapter collected at the end of the book.

Not unexpectedly, in view of the authors' research interests, the chapters entitled "Radical transfer reactions" (18 pages), and "Radical addition reactions" (22 pages) are particularly well done and are a first-class source of facts and current concepts of radical mechanisms. The chapter on "Radicals in biological systems" (15 pages) is also admirable and timely, as this is a growth area in free radical chemistry. Indeed, I would have preferred if this chapter had been somewhat longer, perhaps at the expense of part of that on

"Radical polymerisation" (13 pages). The chapter devoted to "Stereochemistry of free radicals" (9 pages) is also most welcome, as this subject usually receives little attention in textbooks at this level. Awareness of the shape and preferred conformations of radicals is essential if a proper understanding of radical reactions is to be achieved.

Oxidation and reduction in free radical chemistry is a notoriously difficult subject to cover because the terms themselves seem to mean different things to different chemists and the subject matter does not lend itself easily to uniform treatment. Because of this the 15-page chapter on this subject seemed rather short and I would have preferred to have seen a fuller account given.

The book also gives brief introductions to the use of electron spin resonance spectroscopy and CIDNP, and there are chapters on combustion, radical reactions, homolytic aromatic substitution, radical rearrangements, and radical displacement reactions. All of these are sound, although perhaps not quite as good as some of the chapters mentioned earlier.

In summary, I can fully recommend this book as a compact account of present-day free radical chemistry. My criticisms of it are minor and are essentially restricted to the space allocation given to the various subjects.

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Reptilian ear

The Reptile Ear: Its Structure and Function. By Ernest Glen Wever. Pp. 1024. (Princeton University Press: Princeton, New Jersey, and Guildford, UK, 1979.) \$50; £31.30.

The Reptile Ear is a monumental work of outstanding importance, representing more than 25 years of effort by a distinguished scientist. Much of the work described in this book has not been published before.

Detailed studies of the external, middle, and internal ears of 247 species of reptiles were investigated, including 186 species of lizards belonging to 16 families; 19 species of snakes; 14 species of amphisbaenians; 24 species of turtles; 3 species of crocodilians; and the single existing species of rhynchocephalians.

Wever's investigations were both anatomical and physiological, and this greatly enhances the significance of this work. The anatomical studies were carried out by means of serial celloidin sections. It is remarkable that Wever achieved such excellent and accurate detail by a light microscopic method, as his structural interpretations have been corroborated by scanning electron microscopic studies made by other workers.

Wever presents all his anatomical findings by drawings of excellent quality. It would have been helpful to others, however, to have at least some sections represented by photomicrographs.

A great deal of quantitative structural information is presented, such as papilla basilaris lengths, widths, hair cell numbers, basilar membrane width, numbers of rows of hair cells, and so on. Of particular interest is Wever's descriptions and classifications of various types of tectorial membranes.

Wever's evaluation of the functional capacity of these many ears is by use of the cochlear potential method. In many cases, Wever demonstrates increased functional capacity to be related to structural complexities. Such, however, is not always the case.

A great deal of attention is also directed to discussions of the mechanics of hair cell excitation, a problem of salient importance and interest at the moment. The concluding chapter on the biological and evolutionary significance of the reptilian ear is somewhat brief and perhaps properly conservative. Throughout the text, however, Wever indicates structure in relation to phylogeny and evolution. That the reptiles "experiment" widely with many ear components has been well demonstrated and is one of the reasons for the great interest in the reptilian ear.

The author has been careful to describe his working methods in great detail. This information should be of great value to others.

Numerous recent studies have proved the great value of the reptilian ear as an object of study for investigation of the vertebrate auditory system. Wever's

compendium will serve as a valuable and necessary reference and source book for future investigations. **Malcolm R. Miller**

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African mammalogy

Ecology of African Mammals. By M.J. Delany and D.C.D. Happold. Pp. 434. (Longman: London and New York, 1979.) £25.

THIS book, the third contribution to Longman's Tropical Ecology Series covers Africa south of the Sahara. All aspects of the ecology of African mammals, both large and small, are considered resulting in a big book full of concentrated information. The tendency to categorise the text into sections and sub-sections does not make for easy reading but the approach will no doubt be appreciated by students wishing to note essential points. The book is divided into four parts and consists of thirteen chapters. The first part gives a comprehensive account of African mammals in an historical setting and the second concerns the biotic zones dealing in turn with rain forests, savannas, arid zones and mountains. The third part considers life histories, behavioural ecology, physiology and population dynamics; and the fourth section, consisting of only one chapter, examines the interactions between mammals and man.

The book, which is extremely well illustrated, covers the ground admirably and the authors have made a very thorough job of their survey of the literature. They have tried to give a balanced account,

which is not an easy task considering that the coverage of the field by research workers is far from even. On the whole, they have selected wisely and have not been biased by their own particular research interests, which are mainly in small mammals. Their account of the large mammals, being essentially a literature review, inevitably lacks something of the critical insight which an active worker in the field would provide. A few of the examples could have been better chosen, for example Rwenzori National Park (p. 113) is not very representative of its region in terms of the number of large mammal species in view of the fact that six more ungulate species occur in a game reserve less than 100 km to the east. It is also unfortunate that the work of Dasmann and Mossman, now some 20 years old, should be quoted in support of game ranching because the yields proposed were hopelessly optimistic and could not have been sustained for long.

There are few errors in this excellent book and none of great significance. It will prove extremely useful as a university text particularly in Africa but also in Europe and America for, given the rich mammalian fauna of Africa, it can serve as a general textbook of mammalogy. Research workers will also find it of value as a reference book. **S.K. Eltringham**

S.K. Eltringham, formerly Chief Research Officer of the Uganda National Parks, is now Lecturer in Applied Biology at the University of Cambridge, UK.

Modern ionospheric physics

Ionospheric Techniques and Phenomena. By A. Giraud and M. Petit. Pp. 264. (D. Reidel: Dordrecht, The Netherlands, Boston and London, 1978.) Dfl.85; \$38.

IONOSPHERIC physics has rapidly evolved after the International Geophysical Year (1957-58), from the old days during which traditional ionospheric sounders were the main tool, to the new era during which a number of new powerful tools have been introduced to measure basic ionospheric quantities. As one of those who is aware of such progress and yet has difficulty in catching up on modern literature as a non-specialist in this particular field, I am delighted to have this book. It presents an overall view of the new ionospheric

physics. In particular, the ionosphere is properly and accurately recognized as an integral part of the solar-terrestrial system. In this sense, I feel that the title *Modern Ionospheric Physics* would have been more appropriate.

The book provides a well-balanced presentation of the subject, and will serve as a good textbook for a graduate level course in aeronomy as well as an important reference book for ionospheric physicists and magnetospheric physicists. The book will familiarise readers with all modern techniques in investigating the ionosphere, equipping them well in interpreting data taken from such techniques and also in understanding very recent and future articles in this particular field.

Syun-Ichi Akasofu

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Ocean yearbook

Ocean Yearbook 1. Edited by Elisabeth Mann Borgese and Norton Ginsburg. Pp. 890. (Pacem in Maribus/International Ocean Institute/University of Chicago Press: Chicago and London, 1979.) £17.50; \$25.

ELISABETH Mann Borgese, International Ocean Institute, Malta, and Norton Ginsburg, University of Chicago, are to be congratulated on getting their latest brainchild, the *Ocean Yearbook*, off the ground with its first edition, recently published. Although named a yearbook, the year in question has not been identified; however, the reports of the various organisations which appear in an Annex, cover 1976 and, in certain cases, to mid-1977.

Following a short Editors' Preface, *Ocean Yearbook 1* contains a series of excellent topical articles on Issues and Prospects; Living Resources; Non-living Resources; Transportation and Communication; Marine Science and Technology; Environment; Coastal Management; Military Activities; and Regional Development. These are followed by a number of appendices covering reports from organisations, selected documents and proceedings, a directory of institutions, tables related to the articles, and an index.

The *Yearbook* is a long-awaited product of the International Ocean Institute, Malta, which is described in the Preface as "an offspring of an initiative of the Center for the Study of Democratic Institutions in Santa Barbara, California, USA". Publication of an *Ocean Yearbook* is the logical outcome of the present greatly increased interest in the Oceans which has been kindled by the Third United Nations Conference on the Law of the Sea. It is therefore vitally necessary to follow the developments which are taking place at the Conference, as presented in an excellent critique of the Informal Composite Negotiating Text (1977) by Arvid Pardo, who, in 1967, speaking as the Ambassador of Malta to the United Nations General Assembly, was originally responsible for drawing the attention of the world's governments to the potential of the oceans and their importance to the future of mankind.

Although the key to most other activities in the oceans, there is only one article on Marine Science (and Technology), Perspectives on the Sciences of the Sea by Lord Ritchie-Calder, who sounds a well-justified note of warning in his final two paragraphs. As stated by Sir Francis Beaufort, Hydrographer of the Navy (1829-1855), "The natural tendency of men is to undervalue what they cannot understand". The editors, by their imbalance of content in favour of applied

activities at the expense of basic science, are giving credence to this unfortunate tendency which is particularly prevalent today in the United Nations system and amongst the national aid agencies.

I would also suggest that the selected Documents and Proceedings, contained in Appendix B, should be restricted to "a selection of international agreements, legislation and proceedings of international conferences" which have been finalised. Despite its relevance to Arvid Pardo's article, I query the desirability of reproducing in full the UNCLOS Informal Composite Negotiating Text which had already been replaced by a revised edition before the *Yearbook* was published.

The errors I have noted are minimal. This is a well compiled and well edited publication. My main fear is that the editors have set their sights too high and will be unable to maintain their

present standard and the sheer volume of information contained in the first edition; in any case, at yearly intervals as its name implies. I note only that in the bibliography to Lord Ritchie-Calder's article, Frye, P. is presumably Paul M. Fye, now President of the Corporation, Woods Hole Oceanographic Institution. I must also inform Tom Busha and James Dawson that the only flaw I can find in their most informative article on that excellent organisation IMCO is that the Hydrographic Department of the Admiralty was founded in 1795 (not 1759) when Alexander Dalrymple, Hydrographer to the East India Company, was appointed the first Hydrographer of the Navy.

Desmond P.D. Scott

Desmond P.D. Scott is Secretary of the Intergovernmental Oceanographic Commission, Unesco, Paris.

Magnetohydrodynamic stability

MHD Instabilities. By G. Bateman. Pp. 263. (MIT Press: Cambridge, Massachusetts, and London, 1979.) £15.75; \$22.50.

DESPITE the generality of its title this book addresses rather specifically the question of magnetohydrodynamic stability as it relates to the currently most promising approach to magnetic confinement of fusion plasmas, the Tokamak. This is perhaps surprising in a book the content of which is primarily didactic. However, the result is a relatively compact book which maintains coherence and covers much ground that is of fundamental importance to the subject.

Dr Bateman begins from a virtually axiomatic statement of the governing MHD equations, moderated by a brief enumeration of effects neglected by the MHD formulation. He then proceeds by way of an illustrative chapter on the Rayleigh-Taylor Instability to a development of the theory of equilibrium and linear stability and a derivation of the energy principle. Cylindrical and toroidal geometries are treated and then high beta questions. Non-linear evolution and resistive instabilities each have a chapter. All this theory is sandwiched between two experimental chapters: the first serving as an introduction and motivation, the last as a comparison between theory and experiment.

Although the mathematical details are included in several cases, the emphasis throughout is on understanding the physical concepts. It is refreshing to read so clear an exposition of the physics in a subject which is so often obscured by a tangle of algebra or mystified by the ubiquitous computer 'simulation'. Not that these are absent, but they are treated

with a light, often qualitative, touch, drawing frequently on references to the literature. This approach may feel unsatisfactory for some and it disqualifies the book from being a true reference volume or probably even a self-contained text. However, it greatly enhances its value as an introduction to the subject, and the references (many commendably recent) are there for those who want something heavier to chew on. The provocative questions scattered through the text are great fun.

A serious attempt is made to relate the theory to the experimental context but the connection is not always made really clear. This reflects partly the state of the subject but also an inherent ambivalence in the book between a presentation of fundamentals and a review of current Tokamak MHD research. Given that problem, it seems a pity that Dr Bateman did not choose a rather broader spectrum of applications, to include at least other related toroidal plasma confinement schemes, such as the reversed field pinch in which MHD instabilities are of at least as great importance as in Tokamaks.

MHD Instabilities serves as an excellent introduction for students and researchers in magnetic confinement MHD theory. Its treatment of Tokamak-related problems should prove of great value to the growing numbers in Tokamak research, many of whom are not directly involved in MHD research as such but for whom MHD stability is vital in determining the plasma environment.

I.H. Hutchinson

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obituary

Michael Abercrombie

MR MICHAEL ABERCROMBIE FRS, Director of the Strangeways Research Laboratory, Cambridge, who was distinguished for his contributions to the study of cell locomotory behaviour and malignant invasion in tissue culture, died of cancer on 28 May 1979 after a protracted illness. The importance of his scientific achievement lies as much in his almost single-handed establishment of new general approaches to the problems of development and malignancy as in the details of his own work. His good-humoured courtesy, fairness and deep humanity captured the devotion of all he met throughout the world.

Michael Abercrombie was born on 14 August 1912, at Rhyton Dymock, Gloucestershire. His father, Lascelles Abercrombie, was a distinguished poet and literary critic who later became the Goldsmith Reader at Oxford. In 1931 Michael Abercrombie obtained a Hastings scholarship to read zoology at Queen's College, Oxford. One of his tutors there was Gavin de Beer, an immensely erudite descriptive embryologist, who stimulated his interest in experimental embryology. He left Oxford in 1934 with a 1st in zoology and moved to the Strangeways Laboratory in Cambridge to work with C.H. Waddington who had a dazzling and fascinating influence on him. Here he experimented on the primitive streak in early blastoderms and, for this work, he obtained an Oxford BSc which was then a research degree.

Shortly before the war, he met M.L. Johnson (Jane) at Birmingham University and they were soon married. They spent the war years working together on the degeneration and regeneration of peripheral nerve, a programme of work supervised by J.Z. Young and chosen because of its possible usefulness to wartime surgery. This work was largely carried out in the Anatomy Department at Birmingham but the laboratory was not equipped and Michael had to surrender his superannuation in order to raise money for apparatus. I believe that at the same time he sold his wife's car; her sacrifice surpasses that of Faraday's wife whose linen gown was shredded into strips in order to insulate a coil!

After the war, he took up a lectureship in the Anatomy Department at University College London where J.Z. Young held the chair and here he became reader in charge of the Sub-department of Embryology and eventually, in 1959, a second chair was created for him within the

department. Under his leadership, the sub-department soon became a major centre for embryology in Britain and out of it came the *Journal of Embryology and Experimental Morphology*. In 1962 he became Jodrell Professor of Zoology at University College and, in 1970, he succeeded Honor Fell as Director of the Strangeways Laboratory from which he was to retire in the autumn of 1979.

Michael Abercrombie was a founder member of the London Embryologists' Club which first met in November 1947 and which later became the British Society for Development Biology. He was also elected a Fellow of the Institut International d'Embryologie in 1956, a Fellow of the Royal Society in 1958 and was awarded an honorary doctorate by the University of Uppsala in Sweden in 1977.

The main theme of Abercrombie's research was a study of how signals which control locomotory behaviour are communicated between cells. He early noticed that the Schwann cells associated with regenerating peripheral nerve stumps in culture cease to move once the stumps have met and he soon performed the classic experiment of using time lapse cinematography to observe the behaviour of locomoting fibroblasts in a culture consisting of two confronted explants of chick heart tissue. In 1950 he was joined in this work by Joan Heaysman and they started the publication of their results in 1953.

These papers were of enormous importance to biology. They released a powerful new technique — the careful quantitative analysis of cell behaviour — and simultaneously revealed what has since proved to be probably the most important of the behavioural reactions, contact inhibition of locomotion. With Jack Ambrose, Abercrombie made a cytological study of contact inhibition in both normal and malignant cells and the resulting paper became the ninth most highly quoted in cancer in the period 1960-1975. It initiated an interest in the relation of the cell surface to malignancy which swept the cancer research world.

Until his retirement, he continued to work on models of malignant invasion in culture with Joan Heaysman and Pat Stephenson but, in the mid sixties, the emphasis of his research changed to the machinery of cell locomotion and Sue Pegrum, an electron microscopist, joined the group at University College. Later, when I moved with him to the Strangeways Laboratory, this subject became the main interest of our small research group. Among the topics more peripheral to the

central theme, he continued his early primitive streak experiments with Ruth Bellairs, worked on liver regeneration with R.D. Harkness and investigated collagen formation during wound healing with D.W. James.

Michael Abercrombie's scientific ability was impressive. His command of techniques and comprehensive knowledge of the literature were matched only by his imaginative insight, his dogged pursuit of a problem and his high standard of objective evidence. His reluctance to speculate or to place too simple an interpretation on his results often made his papers hard going for the reader and sometimes led to the oversimplification or outright misrepresentation of his work by others.

In scientific discussion, as always, he was just and courteous. He spoke little (in his own opinion he was almost neurotically shy) and preferred to listen, but what he did say was very carefully thought out and well worth close study. He was extraordinarily knowledgeable not only within science (he read a minimum of 50 papers in preparation for each lecture to his students) but also in music, painting, literature and politics, rivalling, although he would never have agreed, the breadth of learning which he so admired in his friends Lancelot Hogben, J.B.S. Haldane and Gavin de Beer. Nevertheless, he remained totally modest and unassuming, never averse to humble tasks, even after attaining international distinction. He was patiently tolerant of shortcomings in others and was acutely sympathetic towards oppressed or unfortunate people, spending much of his time unobtrusively helping them.

These, then, were the qualities which have endeared Michael Abercrombie to so many. Perhaps his most extraordinary achievement is that, without exception, all who knew him well had a profound respect and fond regard for him. He overcame the hardships and endured the irony of his final illness with typical stoicism and good humour.

Graham A. Dunn

Julia Bell

JULIA BELL, FRCP died on 26 April 1979, shortly after her 100th birthday. She was one of the pioneers of human genetics and worked at University College London throughout her life.

Like many human geneticists she came into the subject from mathematics, which she had studied at Girton, and she worked as Karl Pearson's statistical assistant from

1908 to 1914. At about that time K.P. became increasingly aware of the lack of medical expertise in the Galton Laboratory, and it was at his suggestion that Julia Bell qualified as a doctor. Applied statistics and medicine formed the basis of the work for which she is famous, the collections of human pedigrees published under the general title of the *Treasury of Human Inheritance*. This was the first systematic attempt to document the distribution of human disease within families, and so anxious was Karl Pearson that these pedigrees should be most scrupulously recorded that in his 1912 preface he promised that the 'Treasury' would contain 'no reference to theoretical opinions.' In Julia Bell he found a fellow perfectionist with regard to the data, but by the 1920s a few mild opinions were obviously more acceptable. Subsequently Dr Bell wrote analytical articles which are among the classic papers of the subject. Perhaps the most notable are her 1940 paper on consanguinity rates and her 1937 paper with J.B.S. Haldane on the linkage between the genes for colour-blindness and haemophilia. This was the first demonstration 'that the principles of linkage which have been worked out for other animals also hold good for man.'

Dr Bell was on the staff of the Galton Laboratory until 1965 when she officially retired, at the age of 86! Although never Galton Professor of Human genetics herself she outlived the first three, Pearson, Fisher and Penrose, and in her 90s was still sufficiently concerned with the Laboratory to remonstrate with the Galton Professor when she felt it to be necessary! She was a supporter of the Women's Suffrage Movement, but never a militant. She was something rarer, one of the first generation of women to engage in professional scientific research, and to do it supremely well.

Elizabeth B. Robson

N.D. Riley

NORMAN DENBIGH RILEY, CBE, who died on 26 May 1979 at the age of 88 was an eminent British entomologist with a high reputation as a lepidopterist and as a tireless and effective worker in the interests of international entomology.

For nearly a quarter of a century he was head (Keeper) of the Department of Entomology in the British Museum (Natural History), and between the 1930s and 1950s saw it transformed from a dusty insect storehouse into a modern research institution. We may claim for him, as such a modest man would never claim for himself, that he was largely responsible for the high regard in which the Department of Entomology is still undoubtedly held internationally.

Fifty years ago service to one's country

was more esteemed than it is today, and in 1932 when E.E. Austen (a military man himself) retired as Keeper of Entomology he was instrumental in securing Riley's appointment as his successor in preference to F.W. Edwards. Riley had served in the army with distinction for the whole length of the Great War, rising to the rank of Captain, whereas Edwards — the scientifically more qualified man — had been a non-combatant pacifist through his Quaker convictions.

While Riley might have been fortunate at this juncture in his life, his promotion to Keeper was to prove amply justified. He had a taste for administration unusual in entomologists and soon became the architect both figuratively and almost literally (as he did much to plan the present departmental building) of an expanding Department of Entomology. During his Keepership the insect collections grew enormously, often through purchase of specialized collections negotiated by Riley and there were comparable increases in the department staff. Riley himself was a gregarious man, and when his staff became large enough he encouraged more contact amongst them by organizing annual staff dinner dances, which proved a popular innovation in an otherwise rather stuffy museum. During the Second World War he organised the evacuation of large parts of the collection and the library to safe quarters, and their return after the war, although he remained in South Kensington. The CBE was conferred on Riley in 1952.

In his Presidential Address to the Royal Entomological Society, Riley amused his audience by recalling when his small daughter was overhead to say 'My daddy doesn't work — he goes to the Museum.' In this she had caught Riley's character to a nicety, for to him work and pleasure were synonymous, and long after his official retirement in 1955, until his death in fact, he was a regular visitor to his beloved collection of butterflies in the Museum.

Butterflies were Riley's life-long scientific interest, and one that he was able to indulge professionally from the time of his appointment to the Museum staff as an Assistant in 1911 (which followed on the heels of a short period learning entomology while a demonstrator at the nearby Imperial College). It was not until his return from the war in 1919, however, that Riley published his first modest paper, describing some new Brazilian species of butterflies, but from then on he published regularly for over half a century, his bibliography of about 400 works including books, editorials and reviews as well as scientific papers. His scientific contributions to lepidopterology will be assessed in specialist journals, but mention should be made of his book written in collaboration with his friend Lionel Higgins entitled *A Field Guide to the Butterflies of Britain and Europe*: although only published in 1970 this has

already run through several editions and has been issued in nine languages.

Riley lived as a boy in South London, where he was born in the suburb of Tooting on 20 September 1890 and where he was educated at Harlington School, Balham and Dulwich College. A neighbour was Richard South, doyen amongst British Lepidopterists, and it was he who stimulated Riley's childhood interest in butterflies — so effectively as it turned out that Riley even on the eve of his death was discussing butterfly nomenclature with a friend.

Butterflies in spite of, or perhaps because of, their popularity have always had their problems with scientific names, and Riley was inexorably drawn into the intricacies of nomenclature, with the result that he served for 15 years (from 1950 to 1965) on the International Commission for Zoological Nomenclature. As commission Secretary and member of its editorial Committee he was the mastermind behind the current edition of the International Code of Zoological Nomenclature and bore much of the burden of its production.

He was prominent also in international biology through his election to the International Entomological Congresses and his long connection with Congress organization. For a time Riley served as Chairman of the Entomological Section of the International Union of Biological Sciences. Responsibilities of this kind involved him in occasional distant travel, but most of his collecting was done in Europe. At home Riley's energies were poured into Society affairs. He was a Fellow of the Royal Entomological Society for 67 years (elected in 1912). For the last twenty of these, until his death he held the rare distinction of a Life Fellowship. Riley served on the Society's council (1921–23, 1929–30), and as its Secretary (1926–29, 1941–51), Treasurer (1939–40) and President (1951–52).

He belonged to the British Entomological and Natural History Society for an even longer period, having joined as a boy in 1902, and was its President in 1923–24. This Society gave him special links with many amateur specialists who were keen readers of *The Entomologist* a monthly journal largely devoted to Lepidoptera that Riley owned and edited for thirty-six years (1923–1959). A position he relished was that of organizer over many years of the Verrall Supper, an annual gathering of professional and amateur entomologists; in a long life he missed only two of these occasions and was present at the very first in 1912.

Norman Riley enjoyed every moment of a hard-working life and was the perfect exemplar of those qualities that in a more gentlemanly age were laid down for the conduct of British Museum staff — that they should be 'persons of honour, integrity and liberality.' He is survived by his widow, a son and daughter.

Pamela Gilbert

Georgiana M. Bonser

DR GEORGINA M. BONSER (née Duthie), MD (Manchester), FRCP (London), a leading woman doctor engaged in cancer research in England, died on 9 June 1979 at the age of 81.

She was born on 5 May 1898 and studied medicine in Manchester University and at King's College Hospital, London, qualifying in 1920. By 1923, she had been awarded her MD with distinction and won a travelling fellowship which enabled her to spend a year at the Institut Pasteur in Paris. While there, she met her future husband, Kenneth Bonser, an architect. In 1927 she began her long association with Leeds University and its hospitals. Fortuitously, her interests were channelled into cancer research by her chief, Professor Matthew Stewart. She was a woman with a strong sense of purpose who saw two challenges in her life, the problems of cancer and the need for women in the medical profession to establish their rightful place in an essentially male preserve. Dr Bonser's efforts were eventually to be recognised by her election to the chairmanship of the Leeds branch of the British Medical Association, in 1953, and presidency of the Medical Women's Federation in 1959, which speak for her tenacity and political acumen.

But to appreciate her real achievement, it must be recalled that cancer research, when she began, paid the handsome salary of £200 a year and it was necessary for her to take a part-time job in pathology in order to have enough money to live on. She held several posts in the Leeds hospitals and university until she finally became reader in experimental pathology and cancer research and a consultant pathologist at St. James's Hospital and lived to see this hospital translated from a poor law institution to a modern teaching hospital.

Dr Bonser's contribution to knowledge has been in the studies of chemically induced carcinogenesis of the bladder. Although the association of bladder cancer and the dyeing industry had been established in the late nineteenth century, all efforts to induce bladder tumours with the products from the dyeworks or urine of workers affected with bladder cancer were a failure. Dr Bonser, in 1937, reviewed these disappointing results which were somewhat reminiscent of the research for some of the more elusive infective agents some 50 years previously. Hueper's report that the feeding of large doses of β -naphthylamine to dogs produced bladder cancer was the turning point. By 1943, Dr Bonser reported that the following feeding purified β -naphthylamine to dogs for 5 years, bladder cancer could be induced. This helped to remove suspicion from α -naphthylamine and identify one hazard in the dyeing industry.

She soon realised that the dog was too cumbersome and expensive an animal for research and concentrated on developing models in small animals. From this time, progress was much more rapid as the waiting time to see the result of an experiment was then a matter of one to two years. Dr Bonser's team confirmed that 2-acetylaminofluorene when fed to mice was a bladder carcinogen; this was the first in a long series of experiments that resulted in her being an expert on the interpretation of the pathology of the bladder in laboratory animals.

It became clear to Dr Bonser that she needed specialist help; in part it came from Professor L.N. Pyrah, a urological surgeon who helped with surgical techniques in the animals but the whole programme really became most effective when she recruited D.B. Clayson, a young chemist and J.W. Jull, a biologist to help her. There then followed a long haul in which the metabolism of several potential bladder carcinogens was studied and the carcinogenic properties of their various derivatives tested in her systems. An important part of the techniques developed by Dr Bonser's team was the direct implantation of carcinogens in a variety of different vehicles into the bladders of rats and mice. The net result of their efforts over the next 15 years was to put one part of the bladder carcinogenesis story on to a sound scientific basis. Its eventual impact reached far beyond the dyeworks to embrace the workers in rubber and cable making industries who came into contact with β -naphthylamine. Dr Bonser's strength lay in a sense of purpose, her strong discipline and sound knowledge of general pathology as well as her natural clinical practice; in 1961 she was co-author of a book on the pathology of breast cancer.

In the University her research was solidly based on her nose for a good experiment and skill at being able to interpret the results. Many others who have dived in this field had been hopelessly wrong as they could not tell the difference between hyperplasia of the bladder mucosa from that of early carcinoma. One of her last tasks after her retirement was the writing with one of her former pupils, of a chapter on the pathology of the bladder in experimental animals in the handbook produced by the International Agency for Cancer Research.

Today, with the flood gates open to pour money into testing for carcinogens, it is salutary to reflect that in the smoke and grime of pre-war Leeds, this small, determined and formidable woman was quietly laying the corner stones of the work. The combination of her keen mind and dogged persistence was rewarded by achieving a few lasting goals, but what is most important, she was one of those pioneering women scientists ahead of her time. Georgiana Bonser had been a great credit to her University and dealt a nasty jolt to male chauvinism in medicine, both

of which must have given her the satisfaction of a life well spent.

Edward H. Cooper

Sir Peter Venables

SIR PETER VENABLES died at his home in Birmingham on Sunday 17 June 1979. He was 74. His passing leaves a gap in the world of education in Britain that cannot easily be filled.

Peter Venables started his career as a chemist; to use his own words as "a mere chemist." He emerged from Liverpool University in 1925 with a first-class honours degree in chemistry. It was typical of the man, and of his later career, that, despite the quality of his first degree, he should, before embarking on study for a PhD, have spent a year acquiring an Education Diploma. He then spent four years in research, obtaining a PhD in 1928 followed by 2 years of postdoctoral work.

For the next 11 years his career followed the standard pattern of a teacher in higher education. First as a Lecturer and later as a Senior Lecturer in the Leicester College of Technology (now the Leicester Polytechnic) and then as Head of the Science Department at the South-East Essex Technical College he continued to teach and to carry out research in chemistry. But while at Leicester his lifelong interest in the visual arts was stimulated by the close contacts he made with the staff of the College of Art.

But it was in 1941, at the age of 37, that he took the critical step that was to determine the path of his future career. He accepted the post of Principal of the Municipal College at Southend-on-Sea. This was a move into academic administration. From now on Peter Venables was not "a mere chemist"; he had entered the curious world where education and administration are blended. Academic excellence is a necessary passport to enter this world but in it academic work must take second place (and often third or fourth place), yielding to the need to provide leadership to a company of men and women characterised by independence of mind and often of equal independence of action. He had the qualities required for success, an absolute integrity of purpose which he would pursue, as the occasion required, with consummate patience, with a formidable show of strength or even, where necessary, with guile. He had a mischievous wit and could school his countenance to betray no expression whatsoever as he uttered his most outrageous quips. All in all he inspired in his friends and colleagues both an absolute trust and a deep affection, a combination that inevitably makes a leader of men, and a leader he was to be for the rest of his career.

From Southend-on-Sea he moved in 1947 to become principal of the Royal Technical College at Salford. During the next nine years he laid there the foundation upon which, much later, that college was to become the University of Salford. His qualities were recognised more widely by his election as President and later Chairman of the Council of the Association of Principals of Technical Institutions.

In 1956 he moved again, this time to the College of Technology in Birmingham. Here for a decade he presided over its development as the first College of Advanced Technology in the country, of its transition from a CAT to a technological university and, for a further three years as the first Vice-Chancellor of the University of Aston. He was, therefore, deeply involved in the pedagogical and political arguments about the growth of university and technological education throughout a period of almost revolutionary change. He brought to them his wisdom, his vision and his devotion to a humanitarian ideal. His recognition of the need to draw together the interests of industry and education led him to pioneer the sandwich course which found an honourable place in the spectrum of educational provision.

He also found time and energy to give unstinting service as Chairman, President or member of a whole range of other educational institutions. Of particular note, perhaps was the fact that the British Broadcasting Corporation and the Independent Television Authority, if they agreed on nothing else, both chose him to act over the same period as Chairman of their Further Education Advisory Council and Adult Education Advisory Committee respectively — surely a unique distinction in educational broadcasting.

In 1968 he was also Vice-Chairman of the Committee of Vice-Chancellors and Principals and it was while he held this post that he accepted an invitation from Jennie Lee to become Chairman of the Planning Committee of the Open University. It was an inspired choice; but it came as a surprise and a shock to many of his colleagues who did not share his faith in this new educational venture. But it was a venture that called for all the qualities that he had in abundance, humanitarianism, egalitarianism, the desire to harness technology to the service of society. He imprinted these qualities upon the new institution not only during the planning phase but also, as its first Pro-Chancellor and Chairman of Council, during its early formative years. Throughout that period and, indeed, right up to his death, he remained the consultant architect; his was the grand design even though others might build particular bits.

Numerous honours came his way but he remained just the same, a straightforward and uncomplicated chap. For 47 years he was sustained by a very happy and stable

home. His wife, Ethel, a distinguished scholar in her own right, shared his hopes and his motives. Our sympathy goes to her and to the children in their sad loss, a loss that seems as personal to many of us as it must be to them.

Walter Perry

H.B.D. Kettlewell

HENRY BERNARD DAVIS KETTLEWELL died on 10 May 1979, aged 72. He was one of those dedicated scientists who gave up work as a medical practitioner in order to conduct biological research, so that his professional life falls into two parts.

He was the son of Henry Kettlewell and Kate Davis. In 1936 he married Hazel Margaret, daughter of Sir Frank Wiltshire, having one son and one daughter.

He was educated at Charterhouse and Caius College, Cambridge. He obtained the degrees of MA, MB, and B Chir, and acquiring the status of MRCS and LRCP, he held several hospital appointments. During the war of 1939-45 he worked at the Woking War Hospital, subsequently taking up general practice at Cranleigh, Surrey.

In 1949 he emigrated to South Africa, researching for a time on locust control. He also conducted expeditions to the Kalahari, Belgian Congo and Mozambique. Characteristically, he drove his motor car from Cape Town to Alexandria when in 1952 he decided to take up a Nuffield Research Fellowship in genetics in the Department of Zoology at Oxford. Two years later he became a Senior Research Officer, a position he held until his retirement in 1974. He was elected a Fellow of Wolfson College, Oxford and obtained the degree of DSc at Oxford in 1975.

During his research career, he undertook lecture tours in the USA and Canada and visited Brazil for *Life Magazine* on the occasion of the centenary of the *Origin of Species*. He was co-founder of the Rothschild-Cockayne-Kettlewell Collection of Lepidoptera now housed in the Natural History Museum, South Kensington. This is of a unique type, illustrating the genetics of butterflies and moths with full references to the literature accompanying each set of specimens.

Bernard Kettlewell was the finest living ecologist of the Lepidoptera. Moreover, he was blessed with the good luck which ought to attend devoted field workers. On one occasion he found himself surrounded by large numbers of the Bath White butterfly *Pontia daplidice* in South Cornwall and had a specimen of this and of another fabulously rare species the Short tailed Blue *Everes argiades*, in his net at the same time.

It is, in particular, with the study of industrial melanism in the Lepidoptera

that Kettlewell's name will always be associated. That phenomenon involves the most spectacular, though not the most profound, evolutionary change which has ever been witnessed. He showed that over a hundred species are affected by it in Britain alone. The correct interpretation of the subject had been handicapped by the mistakes and failures of others in the past. These included the wholly incorrect explanation, depending on mutation-pressure, put forward by Heslop-Harrison. Moreover, both ornithologists and entomologists had entirely failed to realise that birds hunting by sight may selectively destroy resting moths. It was the triumph of Kettlewell to show by much detailed observation and experiment that the explanation of this occurrence by mutation-pressure is invalid, and to confute those who maintained it by collaborating with N. Tinbergen to produce brilliant cinematograph pictures demonstrating such predation.

Kettlewell was able to analyse the whole subject of industrial melanism with great success, using in particular the Peppered Moth, *Biston betularia*, of which he bred thousands of specimens for study in the field. Moreover, by various recapture methods he obtained definite information on their survival. Such melanism is confined to species that rest exposed, matching their background. Kettlewell demonstrated that the black form survives better in polluted areas but is eliminated in normal countryside. He also studied the genetics of the situation and showed that the melanism is controlled as a polymorphism, with heterozygous advantage.

This work has important ancillary applications, including an impressive demonstration of the evolution of dominance obtained by crossing the British Peppered Moth with a related Canadian species. Also the evolution of a super-gene in the Oak Eggar, *Lasiocampa quercus*. His work on the Lepidoptera extended in many other directions, including the detection of speciation in its early stages due to isolation by distance. He also carried out a remarkable analysis of variation in the Scarlet Tiger Moth, *Panaxia dominula*, conducted partly in the garden of his country house between Oxford and Banbury.

He recorded his research results in many articles. He also wrote several books, the most important being *The Evolution of Melanism*, 1973 (Clarendon Press, Oxford).

His work gained considerable recognition. He was awarded the Darwin Medal of the USSR in 1959, and the Mendel Medal in Czechoslovakia in 1965. Throughout his research career Kettlewell was constantly demonstrating the importance of studying evolution by observation and experiment using the techniques of ecological genetics.

E.B. Ford